

Open Research Online

The Open University's repository of research publications
and other research outputs

Epidemiology and Chemical Control of *Fusarium* Seedling Blight of Winter Wheat (*Triticum aestivum* L.)

Thesis

How to cite:

Hare, Martin Christopher (1997). Epidemiology and Chemical Control of *Fusarium* Seedling Blight of Winter Wheat (*Triticum aestivum* L.). PhD thesis The Open University.

For guidance on citations see [FAQs](#).

© 1997 Martin Christopher Hare



<https://creativecommons.org/licenses/by-nc-nd/4.0/>

Version: Version of Record

Link(s) to article on publisher's website:

<http://dx.doi.org/doi:10.21954/ou.ro.0000fe7d>

Copyright and Moral Rights for the articles on this site are retained by the individual authors and/or other copyright owners. For more information on Open Research Online's data [policy](#) on reuse of materials please consult the policies page.

oro.open.ac.uk



UNRESTRICTED

**EPIDEMIOLOGY AND CHEMICAL CONTROL OF
FUSARIUM SEEDLING BLIGHT OF WINTER WHEAT
(*Triticum aestivum* L.)**

MARTIN CHRISTOPHER HARE BSc. (Hons), PgD.

**A thesis submitted in partial fulfilment of the requirements of the Open
University for the degree of Doctor of Philosophy**

August 1997

**Harper Adams Agricultural College in collaboration with Zeneca Crop
Protection**

Date of award: 22nd December 1997

ProQuest Number: C645522

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest C645522

Published by ProQuest LLC (2019). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

ACKNOWLEDGEMENTS

I wish to thank my supervisors Dr D. W. Parry and Dr T. F. Preece for their invaluable guidance during the course of this study, Dr T. R. Pettitt for his support and encouragement and Dr S. Abeyasekera for her assistance with some of the statistical analyses performed.

This study was jointly funded by Zeneca Crop Protection and Harper Adams Agricultural College. I wish to thank Dr R. A. Noon and all those at Zeneca for their assistance and all of my friends and colleagues at Harper Adams for their support.

My special thanks go to Helen for her continued support and understanding.

ABSTRACT

Fourteen seed lots of winter wheat were tested for *Microdochium nivale* contamination using various methods. The seed lots tested were largely free from other fungal contaminants and *M. nivale* was both superficial and deep seated.

No relationship between seed weight and *M. nivale* contamination was shown. *Microdochium nivale* contaminated seeds were evenly distributed by weight throughout the three seed lots tested and no relationship between seed appearance and contamination was shown.

Seedling disease symptoms were greatest for *M. nivale* in cold dry conditions and for *F. culmorum* in warm dry soil conditions. Good linear relationships were shown between disease severity and the rate of seedling emergence for both artificially inoculated and naturally contaminated seed. Contamination by *M. nivale* affected seedling vigour as measured by the rate of emergence. Seedlings took longer to emerge as the percentage of *M. nivale* contaminated seeds increased.

Microdochium nivale var. *majus* was shown to be the most common sub-group of *M. nivale* isolated from wheat seed. Of 91 *M. nivale* isolates tested 85 were identified as *M. nivale* var. *majus* and 6 as var. *nivale*, with the var. *majus* isolates being more pathogenic towards wheat seedlings.

The efficacy of fungicide seed treatments was not affected by inoculum load as measured by the percentage of contaminated seed. However, reduced temperature did decrease the efficacy of one of the fungicides tested. In the field, effects on crop emergence and infection were observed following seed treatment at two sites. At one site disease control resulted in an increase in grain yield of 300%.

It is clear that seedling disease will be severe when a seed lot with a high percentage of contaminated seeds is sown into conditions where seedling emergence will be slow. Under such conditions the use of a robust seed treatment is advised.

	Page No.
ACKNOWLEDGEMENTS	i
ABSTRACT	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	xiii
LIST OF FIGURES	xv
LIST OF APPENDICES	xxi
LIST OF PUBLICATIONS	xxi

TABLE OF CONTENTS

CHAPTER 1 Introduction and Literature Review

<u>INTRODUCTION AND LITERATURE REVIEW</u>	2
The Genus <i>Fusarium</i>	2
Diseases of Wheat	5
Sources of Inoculum for Seedling Blight	8
Soil-borne inoculum	8
Seed-borne <i>M. nivale</i> and <i>Fusarium</i> spp. inoculum	11
Wheat seed contamination by <i>M. nivale</i> and <i>Fusarium</i> spp.	12
The occurrence of contaminated wheat seeds	13
Inoculum load on individual wheat seeds	14
Invasion of Wheat Seedlings by <i>M. nivale</i> and <i>Fusarium</i> spp.	15
Environmental Conditions Affecting the Severity of <i>Fusarium</i> Seedling Blight	16
The Effect of <i>Fusarium</i> Seedling Blight on Yield	20
Chemical control of <i>Fusarium</i> seedling blight	21

iii

CHAPTER 2 General materials and methods

MATERIALS AND METHODS 27

 Pathogens used in Experiments 27

 Host Cultivars 27

 Culture of Host 27

 (i) Seed surface sterilization 27

 (ii) Seedling growth media 28

 (iii) Planting of seed 28

 Culture of Pathogen 28

 (i) Aseptic techniques 28

 (ii) Storage of pathogen isolates 30

 (iii) Production of spores 30

 (iv) Preparation of spore suspensions 31

 Inoculation of Seed 32

 Isolation from Plant Material 32

 (i) Seed 32

 (ii) Seedling stem-bases 32

 (iii) Wheat plant stem-bases 33

 Identification of Isolates 34

 (i) Colony characteristics and spore morphology 34

 (ii) Molecular classification of *M. nivale* isolates into the sub-
 groups *M. nivale* var. *nivale* and *M. nivale* var. *majus* using a
 random amplified polymorphic DNA (RAPD) assay 34

 Assessment of the Rate of Seedling Emergence 35

 Disease Assessment 35

 Soil Water 36

 (i) Determination of soil water content 36

 (ii) Determination of soil water retention curve 37

CHAPTER 3 Comparison of methods for the detection of *Microdochium nivale* contamination of winter wheat seed

INTRODUCTION	39
MATERIALS AND METHODS	41
(i) Comparison of the Paper Towel and Agar Methods for the Estimation of <i>M. nivale</i> Contamination of Winter Wheat Seed	41
The paper towel method	42
The agar method	42
Data analysis	42
(ii) The Effect of Seed Surface Sterilization and the Incorporation of Benomyl into Potato Dextrose Agar on the Recovery of <i>M.</i> <i>nivale</i> from Wheat Seed.	43
Data analysis	44
(iii) The Relationship between <i>M. nivale</i> Contamination and the Appearance of Wheat Seeds	45
Data analysis	45
RESULTS	46
(i) A Comparison of the Paper Towel and Agar Methods for the Estimation of <i>M. nivale</i> Contamination of Winter Wheat Seed	46
(ii) The Effect of Seed Surface Sterilization and the Incorporation of Benomyl into Potato Dextrose Agar on the Recovery of <i>M.</i> <i>nivale</i> from Wheat Seed	46
(iii) The Relationship Between <i>M. nivale</i> Contamination and the Appearance of Wheat Seeds	48
DISCUSSION	50

CHAPTER 4 The relationship between wheat seed weight, contamination by *Microdochium nivale* and seedling disease

INTRODUCTION	55
MATERIALS AND METHODS	56
(i) The Relationship between Natural <i>M. nivale</i> Seed Contamination, the Weight of Individual Wheat Seeds and Germination	56
(ii) The Relationship between <i>M. nivale</i> Seed Contamination, Seed Weight and the Severity of <i>Fusarium</i> Seedling Blight and Subsequent Seedling Growth	58
Assessments of seedling emergence, disease severity and seedling growth	59
Data analysis	59
RESULTS	61
(i) The Relationship between <i>M. nivale</i> Contamination, the Weight of Individual Wheat Seeds and the Germination of Seeds Grown on PDA	61
(ii) The Relationship between <i>M. nivale</i> Contamination, Seed Weight and the Emergence of Wheat Seedlings	68
(iii) The Relationship between <i>M. nivale</i> Seed Contamination, Seed Weight and the Severity of <i>Fusarium</i> Seedling Blight Symptoms	68
(iv) The Relationship between <i>M. nivale</i> Seed Contamination, Seed Weight and Seedling Growth	69
Root length	69
Coleoptile length	69
Shoot length	69
Seedling dry weight per pot	71
DISCUSSION	72

CHAPTER 5

The effect of temperature and soil water on the severity of *Fusarium* seedling blight and the relationship between the rate of seedling emergence and pre- emergence seedling death

INTRODUCTION	77
MATERIALS AND METHODS	80
(i) The Maintenance and Measurement of Soil Water and its Effect on <i>Fusarium</i> Seedling Blight of Wheat	80
Preparation of <i>F. culmorum</i> inoculum	80
Inoculation and sowing of seed	80
Maintenance of soil water	81
Environmental conditions	83
Assessment of the rate of seedling emergence	83
Disease assessment	85
Data analysis	86
(ii) The Effect of Temperature and Soil Water on the Pre-emergence Death of Seedlings Following Inoculation with <i>M. nivale</i> and the Relationship between the Rate of Seedling Emergence and Pre- emergence Seedling Death	86
Preparation of <i>M. nivale</i> inoculum	86
Inoculation and sowing of seed	87
Soil water treatments	88
Environmental conditions	88
Assessment of seedling emergence	89
Data analysis	90
(iii) The Effect of Temperature and Soil Water on the Pre-emergence Death of Seedlings Caused by Natural <i>M. nivale</i> Contamination of Winter Wheat Seed and the Relationship between the Rate of Seedling Emergence and Pre-emergence Seedling Death	90
	vii

Natural <i>M. nivale</i> seed contamination and the sowing of seed	90
Temperature and soil water treatments	91
Assessment of seedling emergence	92
Data analysis	92
RESULTS	93
(i) The Maintenance and Measurement of Soil Water and its Effect on <i>Fusarium</i> Seedling Blight of Wheat	93
(ii) The Effect of Temperature and Soil Water on the Pre-emergence Death of Seedlings Following Inoculation with <i>M. nivale</i> and the Relationship between the Rate of Seedling Emergence and Pre- emergence Seedling Death	95
(iii) The Effect of Temperature and Soil Water on the Pre-emergence Death of Seedlings caused by Natural <i>M. nivale</i> Contamination of Winter Wheat Seed and the Relationship between the Rate of Seedling Emergence and Pre-emergence Seedling Death	101
DISCUSSION	106

**CHAPTER 6 The relationship between temperature and natural
 Microdochium nivale contamination of winter wheat seed and
 the effect on subsequent seedling emergence and seedling
 growth**

INTRODUCTION	112
MATERIALS AND METHODS	112
Sowing of seed	112
Assessments of seedling emergence and seedling growth	114

Data analysis	115
RESULTS	115
(i) The Relationship between the Percentage of Seeds Contaminated by <i>M. nivale</i> , Temperature and the Pre-emergence Death of Seedlings and Subsequent Seedling Growth	115
<i>Microdochium nivale</i> seed contamination and germination potential	115
Seedling emergence	116
Coleoptile length	118
Root length	120
(ii) The Relationship between Natural <i>M. nivale</i> Contamination, Temperature and the Rate of Seedling Emergence	120
DISCUSSION	126

CHAPTER 7 **Seed-borne *Microdochium nivale* var. *nivale* and *Microdochium nivale* var. *majus*; their occurrence on wheat seed and pathogenicity towards wheat seedlings**

INTRODUCTION	132
MATERIALS AND METHODS	134
(i) The Occurrence of <i>M. nivale</i> var. <i>nivale</i> and <i>M. nivale</i> var. <i>majus</i> on Naturally Contaminated Winter Wheat Seed	134
(ii) Pathogenicity of <i>M. nivale</i> var. <i>nivale</i> and <i>M. nivale</i> var. <i>majus</i> Isolates towards Wheat Seedlings	135
Isolates used	135
Inoculation of seed	135
Assessment of seedling emergence	136
Assessment of disease severity on emerged seedlings	136

Assessment of seedling growth	137
Data analysis	137
RESULTS	137
(i) The Occurrence of <i>M. nivale</i> var. <i>nivale</i> and <i>M. nivale</i> var. <i>majus</i> on Naturally Contaminated Winter Wheat Seed	137
(ii) Pathogenicity of <i>M. nivale</i> var. <i>nivale</i> and <i>M. nivale</i> var. <i>majus</i> Isolates towards Wheat Seedlings	138
Seedling emergence	138
Disease score on emerged seedlings	139
Seedling growth	139
DISCUSSION	145

CHAPTER 8 **Chemical control of *Fusarium* seedling blight caused by Natural *M. nivale* contamination of wheat seeds**

INTRODUCTION	150
MATERIALS AND METHODS	151
(i) The Relationship between the Percentage of Naturally Contaminated Seeds (Inoculum Load) and the Efficacy of Fungicide Seed Treatments	151
Seed lots used	151
Seed treatments used	152
Sowing of seed	152
Assessments of seedling emergence and seedling growth	153
Data analysis	154
(ii) The Effect of Temperature on the Efficacy of Fungicide Seed Treatments	154

Seed lots and fungicide seed treatments used	154
Assessments of seedling emergence and seedling growth	155
Data analysis	155
(iii) The Efficacy of Fungicide Seed Treatments in the Field	157
Seed lots and fungicide seed treatments used	157
Trial site details and sowing of seed	158
Assessments performed on field trials	158
Data analysis	161
RESULTS	161
(i) The Relationship between the Percentage of Naturally Contaminated Seeds (Inoculum Load) and the Efficacy of Fungicide Seed Treatments	161
Seedling emergence	161
The rate of seedling emergence	162
Seedling coleoptile length	162
Seedling shoot length	165
Seedling root length	165
Seedling dry weight	168
(ii) The Effect of Temperature on the Efficacy of Fungicide Seed Treatments	171
Seedling emergence	171
Rate of seedling emergence	171
Seedling coleoptile length	173
Seedling stem-base infection by <i>M. nivale</i>	174
Seedling disease severity	178
(iii) The Efficacy of Fungicide Seed Treatments in the Field	180
Crop establishment	180
Tiller number per plant	181
Crop ear count	181

Grain yield and moisture content	182
Plants with <i>M. nivale</i> lesions on the stem-base	182
Stem-base infection by <i>M. nivale</i> and <i>F. culmorum</i>	186
DISCUSSION	189

CHAPTER 9 General Discussion

DISCUSSION	198
Source of Inoculum	198
The Effects of the Temperature and Soil Water on <i>Fusarium</i> Seedling Blight	201
Control of <i>Fusarium</i> Seedling Blight of Wheat	201
PROPOSED FURTHER STUDIES	204
REFERENCES	206

LIST OF TABLES

Table	Title	Page
1	Pathogens that commonly cause the <i>Fusarium</i> group of diseases of cereals in the UK.	4
2	Pathogens used in the experiments and the origin of isolates.	27
3	Cultivar, pathogen, source and harvest year of naturally contaminated winter wheat seed used during the work.	29
4	Details of the containers used and their dimensions, the types of compost used and the seed planting rates.	31
5	Key used to assess the severity of <i>Fusarium</i> seedling blight symptoms caused by <i>Microdochium nivale</i> , on wheat seedlings.	36
6	Code number and cultivar of the batches of winter wheat seed used.	43
7	List of treatments used to investigate the effects of seed surface sterilization by sodium hypochlorite (NaOCl) and potato dextrose agar (PDA) amended with benomyl on the recovery of <i>Microdochium nivale</i> from winter wheat seed.	44
8	List of categories to which individual seeds were assigned following visual examination.	45
9	Mean number of <i>Microdochium nivale</i> isolates recovered per Petri dish from winter wheat seed on potato dextrose agar (PDA) or PDA amended with benomyl (10 mg/l) following pre-treatment with sodium hypochlorite (NaOCl) or water (Table 2).	50
10	The percentage of <i>Microdochium nivale</i> contaminated seed in each of the seed appearance categories for seed lots 15, 18, and 19 (Table 3).	49
11	Treatment numbers, seed lot number, mean seed weight of winter wheat seed and fungicide treatment used in the experiment.	60
12	The relationship between the weight of <i>Microdochium nivale</i> contaminated wheat seeds (Table 3) and guazatine seed treatment with respect to their effect on the emergence and subsequent growth of seedlings at 6°C.	70

13	The maximum weight to which trays were watered and the timing of watering. The controls (treatments 13-24) were watered in the same way as their inoculated treatments.	84
14	Mean and maximum soil matric potentials (ψ_m) produced in John Innes compost number 2 at 20°C using different watering regimes. The controls (treatments 13-24) were watered in the same way as their inoculated treatments.	85
15	The mean soil moisture (ψ_m) produced by each low, medium and high soil water treatment at each temperature.	89
16	Germination of seed batches contaminated with <i>Microdochium nivale</i> at four temperatures. Germination tests were performed on moist filter paper in Petri dishes.	116
17	Base temperature for emergence and thermal time to emergence for six seed lots of winter wheat cv. Riband naturally contaminated with <i>Microdochium nivale</i> (Table 3).	126
18	Isolates of <i>Microdochium nivale</i> var. <i>majus</i> and var. <i>nivale</i> * from wheat seed listed according to location.	138
19	Details of fungicides used as seed treatments.	151
20	Treatment numbers for treatments used to investigate the relationship between inoculum load and the efficacy of fungicide seed treatments for the control of <i>Microdochium nivale</i> seedling blight of winter wheat.	153
21	Treatment numbers for treatments used to investigate the relationship between temperature and the efficacy of fungicide seed treatments for the control of <i>Microdochium nivale</i> seedling blight of winter wheat seed.	156
22	Treatment number and fungicide seed treatment used in field trials.	157
23	Site details for the two seed treatment efficacy field trials.	158
24	Assessment details for field trials No. 1 and 2.	160
25	Plant count, tillers per plant, ear count and grain yield at trial site No. 1 (Table 24).	183
26	Plant count, tillers per plant, ear count and grain yield at trial site No. 2 (Table 24).	184

LIST OF FIGURES

Figure	Title	Page
1	Generalised disease cycle of <i>Fusarium</i> on small grain cereals (from Parry <i>et al.</i> , 1994).	7
2	The percentage of <i>Microdochium nivale</i> contaminated seed in 14 lots of wheat seed (Table 3) as estimated by the paper towel and agar methods. Seed lots are ranked according to <i>M. nivale</i> contamination as estimated by the paper towel test.	47
3	Frequency distribution by weight of wheat seed (lot No. 15, see Table 3). <i>Microdochium nivale</i> contaminated and non-contaminated seed in each weight category are presented.	62
4	Frequency distribution by weight of wheat seed (lot No. 19, see Table 3). <i>Microdochium nivale</i> contaminated and non-contaminated seed in each weight category are presented.	63
5	Frequency distribution by weight of wheat seed (lot No. 18, see Table 3). <i>Microdochium nivale</i> contaminated and non-contaminated seed in each weight category are presented.	64
6	The percentage of <i>Microdochium nivale</i> contaminated and non-contaminated wheat seed (lot No. 15, see Table 3) in each weight category that had germinated on potato dextrose agar after 14 days at 15°C.	65
7	The percentage of <i>Microdochium nivale</i> contaminated and non-contaminated wheat seed (lot No. 19, see Table 3) in each weight category that had germinated on potato dextrose agar after 14 days at 15°C.	66
8	The percentage of <i>Microdochium nivale</i> contaminated and non-contaminated wheat seed (lot No. 18, see Table 3) in each weight category that had germinated on potato dextrose agar after 14 days at 15°C.	67

- 9 The effect of decreasing mean soil matric potential (Ψ_m) on the final emergence of wheat seedlings cv. Mercia (lot No. 14, see Table 3) inoculated with *Fusarium culmorum* macroconidia expressed as a % of the controls ($y = 1051x + 235$; $R^2 = 0.92$; $P < 0.01$). Values are means of three replicates. 94

- 10 The relationship between mean soil matric potential (Ψ_m) and the mean rate of seedling emergence for non-inoculated wheat seedlings cv. Mercia (lot No. 14, see Table 3) ($y = 0.0962x + 0.238$; $R^2 = 0.89$; $P < 0.01$). Values are means of three replicates. SE are smaller than symbols. 96

- 11 The effect of mean soil matric potential (Ψ_m) on pre-emergence death (●) ($y = -1007x - 116$; $R^2 = 0.92$; $P < 0.01$) and post-emergence symptoms (○) ($y = 968x + 187$; $R^2 = 0.85$; $P < 0.01$) caused by seed-borne *Fusarium culmorum* infection of winter wheat seedlings cv. Mercia (lot No. 14, see Table 3). Values are means of three replicates; bars indicate SE. 97

- 12 The effect of temperature and soil water potential on the emergence of wheat seedlings (lot No. 14, see Table 3) inoculated with *Microdochium nivale* conidia. 99

- 13 The effect of temperature and soil water potential on the rate of emergence of wheat seedlings (lot No. 14, see Table 3) inoculated with *Microdochium nivale* conidia. 100

- 14 The relationship between the time taken for wheat seedlings cv. Mercia (lot No. 14, see Table 3) inoculated with *Microdochium nivale* macroconidia to emerge and the final number that emerged expressed as a percentage of the controls ($y = -5.046x + 117.5$; $R^2 = 0.92$; $P < 0.01$). 102

- 15 The effect of temperature and soil water potential on the emergence of wheat seedlings (lot No. 15, see Table 3) naturally contaminated with *Microdochium nivale*. LSD ($P = 0.05$) = 0.8; SEM = 0.42; D of F = 108; CV = 20%. 104

- 16 The effect of temperature and soil water potential on the rate of emergence of wheat seedlings (lot No. 15, see Table 3) naturally contaminated with *Microdochium nivale*. LSD ($P = 0.05$) = 0.0038; SEM = 0.00191; D of F = 108; CV = 10.6%. 105

- 17 The relationship between the time taken for wheat seedlings cv. Riband (lot No. 15, see Table 3) naturally contaminated with *Microdochium nivale* to emerge and the final number that emerged ($y = -0.313x + 12.57$; $R^2 = 0.95$; $P < 0.01$). 107

- 18 The effect of (a) temperature (SEM = 0.232), (b) *Microdochium nivale* seed contamination (SEM = 2.85) and (c) The relationship between temperature and natural *M. nivale* seed contamination (SEM = 0.57; LSD ($P = 0.05$) = 1.1) on the emergence of wheat seedlings cv Riband (lot No. 21, 20, 19, 18, 17 and 15, see Table 3). D of F = 96; CV = 16%. 117
- 19 The effect of (a) temperature (LSD ($P = 0.05$) = 2.2; SEM = 1.10) and (b) *Microdochium nivale* seed contamination (LSD ($P = 0.05$) = 2.6; SEM = 1.35) on the coleoptile length of emerged wheat seedlings cv Riband (lot No. 21, 20, 19, 18, 17 and 15, see Table 3), D of F = 96; CV = 12%. 119
- 20 The effect of (a) temperature (LSD ($P = 0.05$) = 5.0; SEM = 2.56) and (b) *Microdochium nivale* seed contamination (LSD ($P = 0.05$) = 6.2; SEM = 3.14) on the root length of emerged wheat seedlings cv Riband (lot No. 21, 20, 19, 18, 17 and 15, see Table 3). D of F = 96; CV = 17%. 121
- 21 The relationship between temperature and wheat seedling emergence for six seed lots of winter wheat cv. Riband (lot No. 21, 20, 19, 18, 17 and 15, see Table 3) naturally contaminated by *Microdochium nivale*. 122
- 22 The relationship between the percentage of wheat seeds cv. Riband (lot Nos 21, 19, 18, 17 and 15, see Table 3) naturally contaminated with *Microdochium nivale* and the thermal time to mean seedling emergence ($y = 2.28x^{0.0904}$; $R^2 = 90.2\%$; $P < 0.01$). 124
- 23 The relationship between the time taken for wheat seedlings to emerge and the final emergence of seedlings cv. Riband (lot No. 21, 20, 19, 18, 17 and 15, see Table 3) naturally contaminated by *Microdochium nivale*. 125
- 24 The emergence of wheat seedlings following inoculation of seed (lot No. 4, see Table 3) with conidia from isolates of *Microdochium nivale* var. *majus* and *M. nivale* var. *nivale*. Seedlings were grown at 6°C. The SEM for non-inoculated seed = 0.248 and for inoculated seed = 0.351. The LSD ($P = 0.05$) for non-inoculated vs inoculated = 0.17 and for inoculated vs inoculated = 0.97; D of F = 68; CV = 13%. 140
- 25 The disease score of emerged wheat seedlings following inoculation of seed (lot No. 4, see Table 3) with conidia from isolates of *Microdochium nivale* var. *majus* and *M. nivale* var. *nivale*. Seedlings were grown at 6°C. The SEM for non-inoculated seed = 1.98 ($n = 16$), for inoculated seed with the exception of isolate No. 4 = 2.81 ($n = 8$) and for isolate No. 4 = 7.9 ($n = 1$); D of F = 61; CV = 22%. 141

- 26 The coleoptile length of wheat seedlings following inoculation of seed (lot No. 4, see Table 3) with conidia from isolates of *Microdochium nivale* var. *majus* and *M. nivale* var. *nivale*. Seedlings were grown at 6°C. The SEM for non-inoculated seed = 0.72 and for inoculated seed = 1.01. The LSD ($P = 0.05$) for non-inoculated vs inoculated = 2.5 and for inoculated vs inoculated = 2.9; D of F = 61; CV = 8.5%. 142
- 27 The shoot length of wheat seedlings following inoculation of seed (lot No. 4, see Table 3) with conidia from isolates of *Microdochium nivale* var. *majus* and *M. nivale* var. *nivale*. Seedlings were grown at 6°C. The SEM for non-inoculated seed = 2.76 and for inoculated seed = 3.91. The LSD ($P = 0.05$) for non-inoculated vs inoculated = 9.6 and for inoculated vs inoculated = 11.1; D of F = 61; CV = 8.6%. 143
- 28 The root length of wheat seedlings following inoculation of seed (lot No. 4, see Table 3) with conidia from isolates of *Microdochium nivale* var. *majus* and *M. nivale* var. *nivale*. Seedlings were grown at 6°C. The SEM for non-inoculated seed = 3.27 and for inoculated seed = 4.62. The LSD ($P = 0.05$) for non-inoculated vs inoculated = 11.3 and for inoculated vs inoculated = 13.1; D of F = 61; CV = 14%. 144
- 29 The effect of (a) fungicide seed treatment (SEM = 0.190), (b) natural *Microdochium nivale* seed contamination (SEM = 0.174) and (c) the relationship between fungicide seed treatment and *M. nivale* seed contamination (SEM = 0.43, LSD ($P = 0.05$) = 1.2) on the mean number of emerged wheat seedlings cv Riband (lot Nos 20, 19, 17, 15 and 22, see Table 3). D of F = 60; CV = 5.14%. 163
- 30 The effect of (a) fungicide seed treatment (SEM = 2.5×10^{-4} ; LSD ($P = 0.05$) = 6.8×10^{-4}) and (b) natural *Microdochium nivale* seed contamination (SEM = 2.2×10^{-4} ; LSD ($P = 0.05$) = 6.2×10^{-4}) on the rate of emergence of wheat seedlings cv Riband (lot Nos 20, 19, 17, 15 and 22, see Table 3). D of F = 60; CV = 2.6%. 164
- 31 The effect of (a) fungicide seed treatment (SEM = 0.45), (b) natural *Microdochium nivale* seed contamination (SEM = 0.41) and (c) the relationship between fungicide seed treatment and natural *M. nivale* seed contamination (SEM = 1.0; LSD ($P = 0.05$) = 2.8) on the coleoptile length (mm) of emerged wheat seedlings cv Riband (lot Nos 20, 19, 17, 15 and 22, see Table 3). D of F = 60; CV = 5.5%. 166

- 32** The effect of (a) fungicide seed treatment (SEM = 2.32), (b) natural *Microdochium nivale* seed contamination (SEM = 2.12) and (c) the relationship between fungicide seed treatment and natural *M. nivale* seed contamination (SEM = 5.19; LSD ($P = 0.05$) = 14.4) on the shoot length (mm) of emerged wheat seedlings cv Riband (lot Nos 20, 19, 17, 15 and 22, see Table 3). D of F = 60; CV = 9.4%. **167**
- 33** The effect of (a) fungicide seed treatment (SEM = 4.22; LSD ($P = 0.05$) = 11.7) and (b) natural *Microdochium nivale* seed contamination (SEM = 3.85; LSD ($P = 0.05$) = 10.7) on the root length (mm) of emerged wheat seedlings cv Riband (lot Nos 20, 19, 17, 15 and 22, see table 3). D of F = 60; CV = 17.9%. **169**
- 34** The effect of (a) fungicide seed treatment (SEM = 0.0114), (b) natural *Microdochium nivale* seed contamination (SEM = 0.0104) and (c) The relationship between fungicide seed treatment and natural *M. nivale* seed contamination (SEM = 0.0254, LSD ($P = 0.05$) = 0.07) on the dry weight of emerged wheat seedlings cv Riband (lot Nos 20, 19, 17, 15 and 22, see Table 3). D of F = 60; CV = 8.9%. **170**
- 35** The effect of (a) fungicide seed treatment (SEM = 0.341), (b) temperature (SEM = 0.305) and (c) The relationship between fungicide seed treatment and temperature (SEM = 0.682; LSD ($P = 0.05$) = 1.36) on the emergence of wheat seedlings cv Riband (lot No. 15, see Table 3). D of F = 60; CV = 17%. **172**
- 36** The effect of (a) fungicide seed treatment (SEM = 8.7×10^{-4}), (b) temperature (SEM = 7.79×10^{-4}) and (c) The relationship between fungicide seed treatment and natural *Microdochium nivale* seed contamination (SEM = 1.23×10^{-3} ; LSD ($P = 0.05$) = 3.41×10^{-3}) on the emergence of wheat seedlings cv Riband (lot No. 15, see Table 3). D of F = 60; CV = 3.6%. **175**
- 37** The effect of (a) fungicide seed treatment (SEM = 0.505) and (b) temperature (SEM = 0.452) on the coleoptile length of wheat seedlings cv. Riband (lot No. 15, see Table 3). D of F = 120; CV = 9.2%. **176**
- 38** The effect of (a) fungicide seed treatment (SEM = 0.417), (b) temperature (SEM = 0.373) and (c) The relationship between fungicide seed treatment and natural *Microdochium nivale* seed contamination (SEM = 0.834, LSD ($P = 0.05$) = 2.31) on the infection of wheat seedling coleoptiles cv Riband (lot No. 15, see Table 3) D of F = 60; CV = 33%. **177**

- 39 The effect of (a) fungicide seed treatment (SEM = 4.05) and (b) temperature (SEM = 3.62) on disease severity of emerged wheat seedlings cv Riband (lot No. 15, see Table 3). D of F = 60; CV = 57%. 179
- 40 The number of wheat plants out of ten with *Microdochium nivale* lesions on the stem-base at trial site No. 2 (for details of site, see Table 24) at (a) GS 10-11 (SEM = 0.52; LSD ($P = 0.05$) = 1.6; D of F = 15; CV = 27%), (b) GS 30-31 (SEM = 0.59; LSD ($P = 0.05$) = 1.8; D of F = 15; CV = 28%). 185
- 41 Number of wheat plants out of ten infected by *Microdochium nivale* following treatment with fungicide seed treatments at four times during the growing season (GS 10-11, 30-31, 45 and 75) at trial site No. 1 (for details of site, see Table 24). 187
- 42 Number of wheat plants out of ten infected by *Microdochium nivale* following treatment with fungicide seed treatments at four times during the growing season (GS 10-11, 30-31, 45 and 75) at trial site No. 2 (for details of site, see Table 24). 188
- 43 Number of wheat plants out of ten infected by *Fusarium culmorum* following treatment with fungicide seed treatments at four times during the growing season (GS 10-11, 30-31, 45 and 75) at trial site No. 1 (for details of site, see Table 24). 190
- 44 Number of wheat plants out of ten infected by *Fusarium culmorum* following treatment with fungicide seed treatments at four times during the growing season (GS 10-11, 30-31, 45 and 75) at trial site No. 2 (for details of site, see Table 24). 191

LIST OF APPENDICES

Appendix	Title	Page
1	Degrees of freedom, F ratio and probability values for main factors and interactions in the study of the effects of seed contamination and fungicide seed treatment on <i>Microdochium nivale</i> seedling blight of wheat.	220
2	Degrees of freedom, F ratio and probability values for main factors and interactions in the study of the effects of seed contamination, fungicide seed treatment and temperature on <i>Microdochium nivale</i> seedling blight of wheat.	221
3	F ratio & P values for stem-base infection data from trial sties No. 1 and 2.	222

LIST OF PUBLICATIONS

Hare M.C., Parry D.W. and Noon R.A. (1995) Towards the prediction of *Fusarium* seedling blight of wheat. In: Hewitt *et al.*, eds. *A vital role for fungicides in cereal production*. Oxford: Bios Scientific Publishers Ltd.

Parry D.W, Rezanoor H.N., Pettitt T.R., Hare M.C. and Nicholson P. (1995) Analysis of *Microdochium nivale* isolates from wheat in the UK during 1993. *Annals of Applied Biology*. 126: 449-455.

Hare M.C. and Parry D.W. (1996) Observations on the maintenance and measurement of soil water in simple pot experiments and its effects on seed-borne *Fusarium culmorum* seedling blight of winter wheat. *Annals of Applied Biology*. 129: 227-235.

CHAPTER 1

Introduction and literature review

INTRODUCTION AND LITERATURE REVIEW

The Genus *Fusarium*

According to Booth (1971) the genus *Fusarium* was first described by Link in 1809. *Fusarium* is classified within the order *Deuteromycotina* (Fungi Imperfecti) in the class *Hyphomycetes*. The genus *Fusarium* contains fungi which are characterized by fusoid, curved, septate macroconidia. The distinctive macroconidia form slimy masses called sporodochia on branched conidiophores. Microconidia where formed are smaller, aseptate or uni-septate and ovate in shape. Terminal or intercalary chlamydospores are common in the genus.

There are many species within the genus *Fusarium*. Those commonly occurring on cereals in the U.K. are *F. culmorum*, *F. avenaceum*, *F. graminearum* and *F. poae*. The known teleomorphs of these *Fusarium* spp. belong to the order *Hypocreales*, family *Hypocreaceae* of the *Ascomycotina*. They are classified as members of the genera *Calonectria*, *Gibberella*, *Micronectria* and *Nectria*, all of which are closely related.

Until 1983, *Microdochium nivale* (teleomorph: *Monographella nivalis*), formerly *Fusarium nivale* was considered to be part of the genus *Fusarium* (Samuels and Hallet, 1983). *Fusarium nivale* was reclassified as *M. nivale* owing to its annellidic conidiogenesis. Within *F. nivale*, Wollenwebber (1931) had already distinguished two varieties, *F. nivale* var. *majus* and *F. nivale* var. *nivale*, the former

having larger conidia. This observation was later confirmed by Gerlach and Nirenberg (1982) who recorded that *F. nivale* var. *nivale* was mostly 1-3 septate whilst *F. nivale* var. *majus* was distinguished by wider, predominantly three septate conidia. However, the separation of *M. nivale* into two groups was questioned by Nelson *et al.* (1983) and by Litschko and Burpee (1987) who could not differentiate any distinct types using morphological criteria. Recently, Lees *et al.* (1995) used random amplified polymorphic DNAs (RAPDs) to investigate variability within *M. nivale* from cereals in the U.K. This study revealed extensive variability within *M. nivale* but nevertheless demonstrated the presence of a uniform sub-group which correlated broadly to var. *majus* as determined by conidial morphology. These findings were confirmed among isolates of *M. nivale* from elsewhere in Europe by isozyme analysis and study of polymorphisms within the internal transcribed spacer (ITS) region of rDNA (Maurin *et al.* 1995). However, it was not clear from these studies whether the remaining isolates were members of further sub-groups. In the absence of further information, these isolates were termed var. *nivale* (Maurin *et al.*, 1995) to differentiate them from the homogenous var. *majus* sub-group.

The anamorphs and teleomorphs of the pathogens that commonly cause *Fusarium* diseases of cereals in the U.K. are given in Table 1.

Fusarium spp. occur commonly throughout temperate and tropical regions of the world. *Fusarium* has been isolated from soil and plant material in Africa (Tarr, 1962), Asia (Kelman and Cook, 1977), Australasia (Wearing and Burgess, 1977), Canada (Gordon, 1954 and 1959), Europe (Maric, 1981; Cassini, 1981) and the

United States of America (Cook, 1968 and 1980) although the occurrence of individual species may be determined by local environmental conditions.

Table 1. Pathogens that commonly cause the *Fusarium* group of diseases of cereals in the U.K.

Anamorph	Teleomorph
<i>Fusarium culmorum</i> (W.G. Smith) Sacc.	Not known
<i>Fusarium avenaceum</i> (Fr.) Sacc.	<i>Gibberella avenacea</i> Cook. (not recorded in the U.K.)
<i>Fusarium poae</i> (Peck) Wollenweber	Not known
<i>Fusarium graminearum</i> Schwabe	<i>Gibberella zeae</i> (Schw.) Petch.
<i>Microdochium nivale</i> (Fr.) Samuels & Hallet	<i>Monographella nivalis</i> (Schaffnit) E. Müller

Many *Fusarium* spp. are primary saprophytes which survive on dead and decaying plant material and other organic substrates (Booth, 1971). The predominant interest in *Fusarium* spp. has however occurred due to their importance as plant pathogens. *Fusarium moniliformae*, *F. culmorum*, *F. avenaceum* and *F. graminearum* are all economically important pathogens of graminaceous crops; pakah-boeng of

sugar cane, bakanae diseases of rice and seedling, foot rot and ear diseases of small grain temperate cereals. *Fusarium* spp. also cause wilt diseases, panama disease of bananas caused by *F. oxysporum* being one of the most devastating diseases in the world (Armstrong and Armstrong, 1948).

Diseases of Wheat

Fusarium spp. and *M. nivale* are responsible for three diseases of wheat; *Fusarium* seedling blight, *Fusarium* foot rot and *Fusarium* ear blight (scab).

The first record of disease of cereals in the U.K. caused by *Fusarium* spp. was made in 1884 by Smith. Later, Bennett (1935) found 14 *Fusarium* spp. associated with disease in U.K. cereal crops. However, of these, only five species (Table 1) are usually accepted as primary causal pathogens.

Wheat can be attacked at all stages of its development by all of the pathogens, with the exception of *Fusarium poae*. There is little evidence to suggest that *Fusarium poae* is a pathogen of seedlings (Colhoun and Park, 1964) or stem-bases, although according to Polley *et al.* (1991) *F. poae* was the most common pathogen infecting ears of winter wheat in a survey conducted between 1989 and 1990 in the U.K.

Attack at early stages of wheat development can cause death of seedlings before and after emergence. Latent or subsequent infection can cause foot rot characterised by brown lesions on nodes and internodes. Severe foot rot can result

in “white heads” (bleached ears containing no grain) or lodging. Later on in the development of the crop ears can become infected. One or many florets may become bleached and grain may become shrivelled and contaminated.

The three *Fusarium* diseases of cereals; seedling blight, foot rot and ear blight are shown in the generalised disease cycle of *Fusarium* on small grained temperate cereals (Figure 1). It can be seen that each disease can provide inoculum for future infection during the next stage of the host’s growth cycle. Ear blight through infected seed can provide a source of inoculum for seedling blight (Rennie *et al.*, 1983) and seedling blight can progress into foot rot (Duthie and Hall, 1987). The link between foot rot and ear blight is less clear, although splash dispersal of spores in water has been demonstrated (Jenkinson and Parry, 1994) and the possibility of systemic infection highlighted (Snijders, 1990).

This succession of diseases may however be broken by environmental conditions unfavourable for disease development or by the action of fungicides. However, the ability of these *Fusarium* spp. to saprophytically colonise crop debris means that cereal crops are always under threat from soil-borne inoculum.

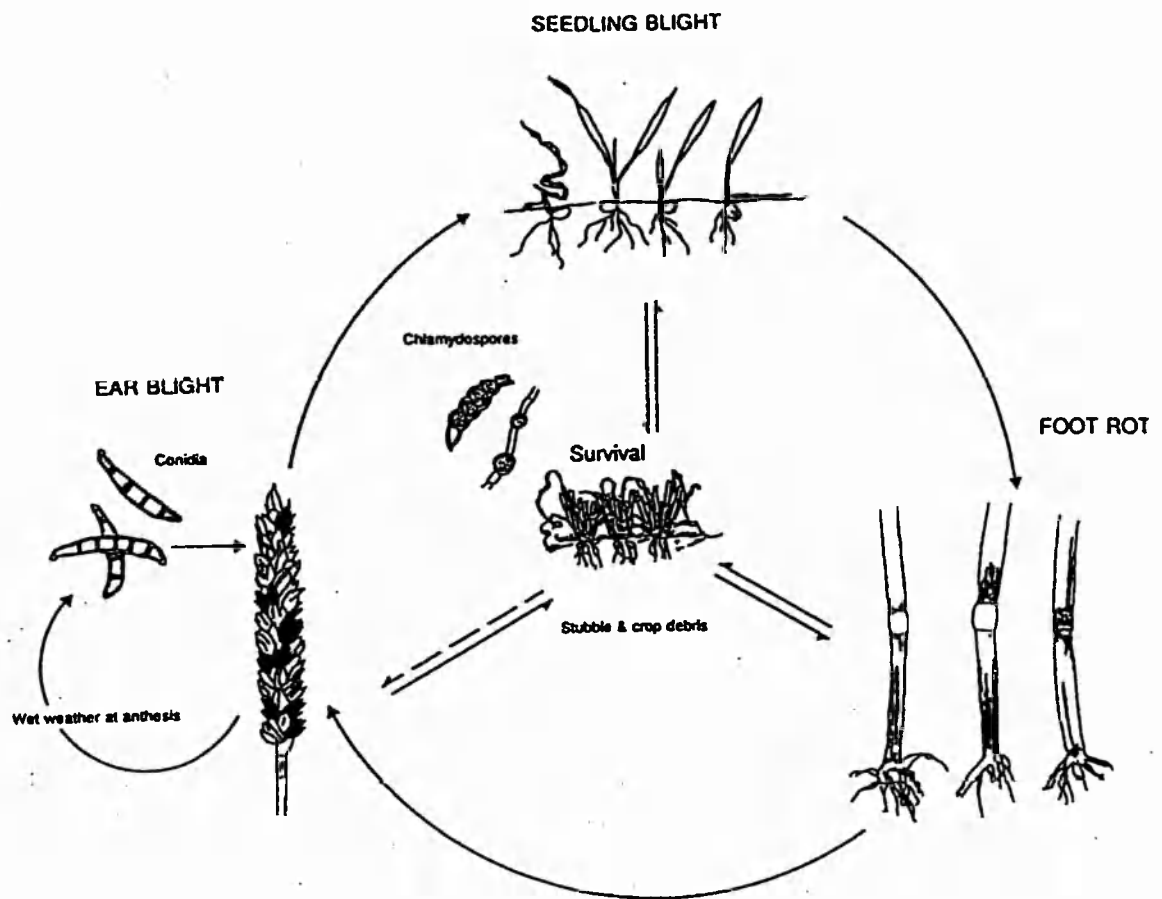


Figure 1 Generalised disease cycle of *Fusarium* on small grain cereals (from Parry *et al.*, 1994).

Sources of Inoculum for Seedling Blight

There are two main sources of inoculum for seedling blight; contaminated seed and soil.

Soil-borne inoculum

Fusarium seedling blight pathogens can survive in soil, although their methods of survival appear to differ. Snyder and Nash (1968) investigated the occurrence of *Fusarium* in field soil at Rothamsted Experimental Station, U.K. The sampled soils included those that had contained continuous cereals or root crops for 100 years or more, as well as those that had been in long-term pasture and woodland. Isolations from these samples, excluding crop debris and organic matter, showed that *F. culmorum* was the only species readily isolated from fields of continuous cereals or long-term pastures. *Fusarium culmorum* was also readily isolated from woodland soils, as was *F. avenaceum*. However, *F. culmorum* was not isolated from fields of continuous root crops. In these fields, *Fusarium oxysporum* was the predominant species.

Resting spores of *F. culmorum* and *F. avenaceum* were found in the above soils. *Fusarium culmorum* readily produces chlamydospores which are thick-walled and long-lived in soil (Cook, 1968), whereas *F. avenaceum* rarely forms true chlamydospores, instead producing a type of resting spore within macroconidia and hyphae, which has been shown to enable the fungus to survive in soil for up to 18

months (Hargreaves and Fox, 1978).

In addition, to its survival as chlamydospores, *F. culmorum* can survive in the soil on crop debris. Sadisivan (1939) readily isolated *F. culmorum* from buried wheat straw. Walker (1942) confirmed Sadisivan's findings and concluded that *F. culmorum* was an important colonizer of organic matter in field soils.

As *M. nivale* is not known to produce resting spores it is not surprising that Snyder and Nash (1968) were unable to isolate *M. nivale* from field soils when excluding organic matter even though it was readily isolated along with *F. culmorum* and *F. avenaceum* from diseased cereal plants. However, the inability to isolate *M. nivale* directly from field soil appears to be more complex than a lack of resting spores.

Gordon (1954) was also unable to isolate *M. nivale* from 1674 soil samples from cereal plots in Canada. In addition, Rawlinson and Colhoun (1969) could not isolate *M. nivale* directly from soil samples, although "bait" plants became infected when grown in these same soil samples. Therefore, although *M. nivale* could not be isolated directly from soil, it was nevertheless there.

It is clear that the precise method by which *M. nivale* persists in soil is not known, although there is evidence to suggest that crop debris in the form of buried straw may be important. Booth and Taylor (1976a) investigated straw as a possible source of inoculum for seedling blight of wheat under field conditions. When naturally infected straw from the previous crop was removed before sowing 47% of seedlings became infected. Where straw was left on the surface and seeds direct

drilled, 70% of seedlings became infected, and when the straw was incorporated by ploughing, 97% of seedlings became infected. In support of this work Booth and Taylor (1976b) showed that *M. nivale* could grow short distances (less than 10 cm) through soil and saprophytically colonise buried straw.

Later work by Al-Hashimi and Perry (1986) failed to support the findings of Booth and Taylor (1976b). Al-Hashimi and Perry found that although *M. nivale* grew through field soil to infect 5% of barley seedlings it was unable to colonise sterile straw or persist on colonised straw in field soil. They concluded that *M. nivale* was only a weak saprophyte and only likely to survive in field soil on straw which had been colonised in the parasitic phase.

The ability of soil-borne inoculum to cause pre-emergence death of wheat seedlings was demonstrated in growth room experiments by Bateman (1977). Following inoculation of the soil with fungus grown on sterilized wheat seed Bateman observed reduced germination and an increase in diseased plants with respect to non-inoculated soil. However, field trials performed by ADAS between 1992 and 1994 using *M. nivale* contaminated and non-contaminated seed stocks of winter wheat with and without a range of fungicide seed treatments produced no evidence to suggest that soil-borne *M. nivale* affected crop establishment or yield in the field. Increases in crop establishment following seed treatment with fungicide were only observed when seed contaminated by *M. nivale* was sown (Paveley *et al.*, 1996).

Seed-borne *M. nivale* and *Fusarium* spp. inoculum

Daamen *et al.* (1991) found that wheat seed contamination by *F. culmorum* and *M. nivale* in the Netherlands, was positively correlated with cumulative precipitation during the months June, July and August. They suggested that wet weather may directly stimulate late ear infection and ear blight disease development. In addition, they stated that delayed crop ripening and harvest could facilitate continued infection leading to contamination of the seed. The effect of atmospheric relative humidity before harvest on the colonisation of wheat seeds in the field by unidentified fungi was studied by Hyde (1950). He found a significant correlation between atmospheric relative humidity at 0900 hrs in what he described as the few weeks before harvest, and the amount of internal fungus in the wheat seeds. The higher the atmospheric relative humidity the greater the amount of internal fungus.

Dickson *et al.* (1921) observed that the contamination of wheat seed by *F. avenaceum*, *F. culmorum* and *F. graminearum* can occur following the inoculation of wheat ears with these pathogens' conidia. They showed that the greatest ear infection occurred at flowering, with initial infection usually occurring through extruded anthers. After the fungus had become established in the anthers it then spread to adjacent tissues.

It has also been suggested that ear infection of wheat may occur from the base of the plant, through systemic growth of the pathogen within the stem. Hutcheon and Jordan (1992) observed ear blight disease symptoms on wheat plants inoculated with *F. avenaceum*, *F. culmorum*, *F. graminearum* or *M. nivale* at the stem-base, prior to

ear emergence. They stated that systemic growth had occurred within the vascular tissue leading to ear disease. However, these workers did not observe fungal colonization of the vascular tissue microscopically. Their conclusion was based solely on isolation of *M. nivale* and *Fusarium* spp. from stem segments at increasing heights from the stem-base.

Wheat seed contamination by *M. nivale* and *Fusarium* spp.

Seed contamination can be the result of the presence of spores on the seed's surface or mycelium within the seed. Hyde and Gallegymore (1951) found many spores of *Fusarium* spp. on the outside of wheat grains harvested from the field in the U. K. These spores were particularly abundant in the beard region. Following surface sterilization to remove surface spore inocula, 1.9% of seeds formed cultures of *Fusarium* spp. on agar plates. After microscopic examination of the seed they found extracellular septate hyphae forming a network on the inner surface of the epidermis. The hyphae were occasionally found to have penetrated the epidermal cells. The sub-epidermal mycelium was finally restricted by desiccation beneath the epidermis during drying of the ripening grain. Elekes (1983) observed hyphae of *F. culmorum*, *F. graminearum* and *F. oxysporum* in the pericarp, most commonly around the embryo, of naturally contaminated wheat seeds from Hungary. Hyphae were also found in the endosperm and testa, but not in the aleurone layer or embryo.

Microdochium nivale was isolated by Bateman (1983), on potato dextrose agar plates, from the epidermis, the pericarp and testa, the endosperm and embryo of

naturally contaminated wheat seeds (cv. Maris Kinsman and Flanders). Although the fungus was distributed throughout the seed, it was most commonly isolated from the epidermis and the pericarp and testa. Sectioning of a few of the infected seeds (cv. Flanders) showed most of the contamination to be in the space below the epidermis and in the crease.

The occurrence of contaminated wheat seeds

Millar and Colhoun (1969) observed that under field conditions in the U.K. when all of the wheat tillers examined were infected by *M. nivale*, a maximum of 30% of the seeds harvested were contaminated by *M. nivale*. In addition, when they artificially inoculated wheat ears with *M. nivale* conidia only 31% of the harvested seeds were contaminated, even though all of the inoculated ears showed disease symptoms. They suggested that this observed maximum percentage (about 30%) was possibly caused by a natural barrier to total grain infection. However, they did not allude to its mechanism.

Wheat seed naturally contaminated by *M. nivale* has subsequently been shown to occur at frequencies greater than 30%. One of the most highly contaminated wheat samples recorded had 79% of its seeds contaminated by *M. nivale* (Humphreys *et al.*, 1995).

The occurrence of wheat seed contaminated by *M. nivale* has increased over recent years. A five year survey of wheat seed in the U.K. begun in 1959 by Hewett (1967) showed that *M. nivale* contamination of wheat seed infection to be 0.3% with

only occasional samples having more than 5%. The 1963 harvest was exceptional, with an average of 5.3% seed infection for samples of the cultivar Cappelle Desprez. Most recently a seed health survey in the U.K. by The Home Grown Cereals Authority (HGCA) and Zeneca showed that 99% of the batches of wheat seed tested between 1992 and 1993 and 68% of those tested in 1994 were contaminated by *M. nivale*.

In contrast to *M. nivale*, natural wheat seed contamination in the U.K. by *F. avenaceum*, *F. culmorum* and *F. poae* has become less common in recent years. A survey of seed-borne *Fusarium* spp. of wheat in the U.K. , between 1959 and 1963, showed that approximately nine out of every ten batches tested were contaminated (Hewett, 1967). The average incidence of contaminated seed was generally low and fluctuated between 2 and 5 %. However, seed contamination in 1960-61 was much higher, with the highest incidence of seed contamination recorded being 29 %. Where seed contamination was high, one species tended to predominate. The most predominant species was usually *F. avenaceum*.

Inoculum load on individual wheat seeds

The effect of increasing inoculum load on the subsequent disease severity in wheat seedlings was studied by Millar and Colhoun (1969). These workers placed increasing numbers of *M. nivale* conidia on the surface of wheat seeds and then observed their effects on subsequent disease severity in wheat seedlings grown in pots in a glasshouse. They found that as the seed spore load increased from 0.5 conidium

per seed to 1 000 000 conidia per seed, the severity of disease increased linearly with log spore load.

The effect of increasing *F. culmorum* inoculum on subsequent seedling blight disease symptoms was investigated by Malalasekera and Colhoun (1969). They too found a linear relationship between log spore load and disease severity in wheat seedlings. These workers then attempted to devise a method by which the extent of individual wheat seed contamination by *F. culmorum* could be assessed. They used a selective medium which inhibited the growth of bacteria and most seed-borne fungi other than *F. culmorum*. Radial growth of colonies derived from contaminated seed was measured after six days, and used as a guide to the degree of contamination. They found a positive relationship between spore load and colony diameter following inoculation of the seed's surface with macroconidia. They concluded that the technique could predict the maximum amount of disease that would occur under conditions favourable for infection. However, they did not state how this could be achieved in practice, and found its application to naturally contaminated seed less effective owing to variability in colony diameter observed from individual seeds.

Invasion of Wheat Seedlings by *M. nivale* and *Fusarium* spp.

According to Bennett (1928) seed and soil-borne inoculum of *F. culmorum* entered wheat through primary root and primary stem. More specifically Simmonds (1928) found that *F. culmorum* infected oats through root hairs, mesocotyl and

coleoptile from conidial germ tubes. Penetration occurred below small appressoria. Hyphae penetrated the cortex of root, shoot and coleoptile, but were rarely observed in tissues within the endodermis. In contrast, appressoria were not observed by Malalasekera *et al.* (1973) when seed-borne inoculum of *F. culmorum* penetrated wheat seedlings through the hypocotyl or coleoptile. Infection by *F. culmorum* of root and coleoptile also was reported by Russell (1931) with penetration occurring frequently between the junction of two epidermal cells. Colonization of roots subsequent to penetration was observed by West (1975) who found that *F. culmorum* hyphae penetrated the stele and entered the xylem of wheat within eight days of inoculation.

There are fewer references to invasion of cereal seedlings by *M. nivale*. Malan (1949) noted that *M. nivale* penetrated the leaves of rye seedlings through their stomata. This method of penetration was also observed by Malalasekera *et al.* (1973) working with *M. nivale* and wheat coleoptiles. They found that following penetration through stomata subsequent intercellular hyphal growth was common. However, penetration of cells by hyphae was rarely observed.

Environmental Conditions Affecting the Severity of *Fusarium* Seedling Blight Symptoms

Many environmental factors are able to affect the severity of seedling blight symptoms. The effect of soil texture on seedling blight symptoms was investigated

by Uoti (1976) who sowed spring wheat seed cv. Ruso artificially inoculated with *F. culmorum* macroconidia into sand, loam and clay soils. Seedling blight severity was greatest in the loam soil with little difference being observed between the sand and clay soils. Sand, silt and clay soils have different characteristics and any one or combination of these may have influenced Uoti's results.

Soils can differ greatly in their ability to provide nutrients to plants. The effect of soil nutrient status on seedling blight severity was investigated by Shen (1940). In pot experiments with wheat seed cv. Little Joss artificially inoculated with *F. culmorum* Shen showed that the severity of disease was lowest when seedlings were grown in conditions of high nutrient status (N, P, and K). Shen also showed that disease symptoms were most severe at low pH (acid soil). This result supports the previous field observations of Blair (1937) who observed a greater incidence of *Fusarium* seedling blight on the more acidic soils in New Zealand. Seedling blight caused by *M. nivale* has also been reported to be most severe in acid soils. Millar and Colhoun (1969) showed in glasshouse pot experiments that wheat seed inoculated with *M. nivale* macroconidia produced the most severely diseased seedlings when grown in the more acid soils tested (pH 4.8 - 8.1).

The factors of soil nutrient status and pH may have affected the results of Uoti previously discussed. However, Uoti attributed the moisture content of the soils as having the greatest influence on seedling blight severity seen. The loam soil being drier than the sand or clay soils tested.

The severity of *Fusarium* seedling blight is known to be affected by

temperature and soil moisture. Early glasshouse studies by Dickson (1923) with *F. graminearum* and wheat seedlings showed that *Fusarium* seedling blight from naturally contaminated and artificially inoculated seed was most severe between 16°C and 28°C. Few disease symptoms were seen above 32°C and none were seen below 12°C. In subsequent field studies he confirmed that disease was most severe in warm soils. No reduction in emergence was seen over the control from early plantings into cool soil conditions in March and April, but only 32% emergence (control 77%) when planted into warmer soil in May.

The effect of soil water on seedling blight of wheat seedlings caused by *F. culmorum* was investigated by Shen (1940). In glasshouse experiments with artificially inoculated seed, Shen showed that the severity of disease symptoms was greatest, in both field soil and sand, at 30% moisture content (as a percentage of saturation) as compared with 50% and 80% moisture content.

More recent studies have addressed the combined effects of temperature and soil water on *Fusarium* seedling blight. The effects of temperature and soil water on seedling blight caused by *F. culmorum* was studied in glasshouse pot experiments using artificially inoculated seed by Colhoun and Park (1964). These workers reported that the percentage of seedlings which died both before and immediately after emergence, as well as the percentage of plants which eventually showed visible lesions, increased as the soil became warmer and drier. Seventy eight percent of seedlings did not emerge in dry (37% maximum water holding capacity (MWHC)) warm (22.5°C) soil, whereas only 2% failed to emerge at 60% MWHC and 13.4°C.

Further work conducted by Colhoun *et al.* (1968) to investigate the additional effects of soil pH, depth of sowing and inoculum density on seedling blight caused by *F. culmorum* also showed that warm temperature (23°C) and dry soil (36% MWHC) were important factors in increasing disease severity.

The effect of temperature and soil water on *Fusarium* seedling blight caused by *M. nivale* has been shown to be different to that of seedling blight caused by *Fusarium* species. According to Colhoun and Park (1964) disease caused by *F. culmorum*, *F. graminearum* and *F. avenaceum* was most severe in warm dry soil, whereas Millar and Colhoun (1969) showed that disease caused by *M. nivale* was most severe in cold dry soil. Millar and Colhoun investigated the effect of temperature and soil water on seedling blight caused by *M. nivale* in glasshouse pot experiments using artificially inoculated seed. They grouped all observed disease symptoms together to create a disease index which proved to be greatest in cold (6.1°C) dry (8.8% moisture content) soil as compared with warm (16.4°C) wet (24.5% moisture content) soil. The effect of low temperature and soil water on pre-emergence death of seedlings artificially inoculated with *M. nivale* was investigated by Duben and Fehrmann (1979). They showed that 91% of seedlings emerged at 20°C and 60% MWHC, whereas only 33% of seedlings emerged at 5°C and 49% MWHC.

The effect of temperature and soil water on the severity of seedling blight symptoms has been linked to the effect of temperature and soil water on the relationship between the pathogen host growth rate ratio and also the coefficient of

velocity of emergence (CVE) of seedlings. Leach (1947) studied the relationship between the pathogen:host growth rate ratio and disease severity for *Pythium ultimum*, *Rhizoctonia solani* and *Phoma betae* with respect to peas, sugar beet, watermelon and spinach under environmentally controlled conditions. He concluded that all other factors being constant, the relative growth rates of the host and the pathogen as affected by temperature, determined to a considerable degree the severity of pre-emergence infection. This theory was applied to the relationships between the pathogen host growth rate ratio and the severity of seedling blight symptoms caused by *F. culmorum* by Malalasekera and Colhoun (1968). These workers primed wheat seeds by soaking them in water prior to inoculation and sowing to simulate the increased rate of seedling emergence seen in warmer, wetter soils. Seeds soaked for three hours prior to inoculation and planting had a lower (39.7) pathogen host growth rate ratio than unsoaked seeds (47.6) and subsequent seedling emergence was higher, 93% and 33% respectively. The CVE of seedlings from soaked seeds was also higher than that from unsoaked seeds, 23.04 and 22.12 respectively.

The Effect of *Fusarium* Seedling Blight on Yield

A reduction in seedling number caused by *Fusarium* seedling blight can result in reduced grain yield in wheat. Colhoun (1970) observed large reductions in seedling emergence in the field following sowing of wheat seed artificially inoculated with *F. culmorum* and *M. nivale*. At harvest yield reductions of up to 20% for *F. culmorum*

and up to 18% for *M. nivale* inoculated seeds were recorded. These yield reductions are modest in comparison to those observed by Uoti (1976). Using spring wheat seed artificially inoculated with *F. culmorum* Uoti observed yield reductions of up to 80% in the field. Yield reductions caused by *M. nivale* in commercial crops occur but are difficult to quantify. However, under normal field conditions yield reductions of up to 40% in Ireland (Humpreys *et al.*, 1995) and 45% in the U.K. (Noon and Jackson, 1992) have been observed in trial plots sown with seed naturally contaminated by *M. nivale*.

Diseased seedlings may have the potential to reduce yield by acting as a source of inoculum for foot rot. Daaman *et al.* (1991) in their survey of foot rot diseases in the Netherlands found that foot rot symptoms caused by *Fusarium* spp. and *M. nivale* were positively but not significantly correlated with average percent seed contamination. However, little is known about the relationship between *Fusarium* foot rot and yield which makes estimation of potential yield loss from seedling blight inoculum difficult.

Chemical control of *Fusarium* seedling blight

Chemical control of *Fusarium* seedling blight has been practised for many years. One of the first treatments to be used against *Fusarium* spp. was chlorophenol-quicksilver (Remy and Vasters, 1914). Subsequently treatments containing copper carbonate, nickel sulphide, iodine-infusorial earth and organo-mercury compounds

have been used to disinfect the seeds' surface, and of these organo-mercury was the most effective (Macheck and Greaney, 1935). Macheck and Greaney showed that organo-mercurial treatments significantly increased the yield of wheat when treated seed was sown in soil heavily infested by *F. culmorum*. The ability of organo-mercury seed treatments to control soil-borne *M. nivale* has also been demonstrated (Bateman, 1977). Bateman showed that concentrations of phenyl mercuric acetate (PMA) from 1.0 to 3.0 $\mu\text{g seed}^{-1}$ progressively increased emergence of wheat sown in soil inoculated with *M. nivale*. Organo-mercury seed treatment has also been shown to be effective against seed-borne inoculum with increases in yield from contaminated seed being observed in oats (Richardson, 1974); in barley (Clark, 1981) and wheat (Rennie *et al.*, 1990).

However, according to Millar and Colhoun (1969) control of seed-borne *Fusarium* seedling blight of wheat when using organo-mercury was not complete when treating naturally contaminated seed. Bateman (1976, 1983) also observed incomplete control with PMA and stated that the failure to control *M. nivale* with organo-mercury had probably resulted from the pathogen being exceptionally deep-seated. Following dissection of a small number of seeds Bateman isolated *M. nivale* from the endosperm and embryo.

Incomplete control of seed-borne *Fusarium* spp. has also been observed using non-mercurial seed treatments. Scheinpflug and Duben (1988) showed that *Fusarium* mycelium in the pericarp of wheat seed could be controlled by a range of non-mercurial fungicides. However, they considered that if the infection had penetrated

deeply and had damaged the embryo, a fungicide with systemic activity would be considerably better than a protectant although even with a systemic compound complete control could not be completely guaranteed.

The use of mercury seed treatments in commercial crops in the U.K. was commonplace (Rennie *et al.*, 1980). However, following implementation in 1992 of the EC commission directive 79/117/EC (Anon., 1979) mercury-containing seed treatments have not been registered for use in the U.K. Seed treatments containing compounds from the demethylation inhibitor (DMI) and benzimidazole (MBC) groups had previously been used as alternatives to mercury with many workers suggesting that fungicides from the DMI and MBC groups should be used in mixture to achieve commercially acceptable control of *M. nivale* seedling blight of winter wheat in the field. Froberger (1978) recommended that triadimenol, a DMI, should not be used alone for the control of *Fusarium* seedling blight of cereals. This view was supported by Wainwright *et al.* (1979) who showed that although triadimenol alone gave useful control of pre-emergence seedling death caused by seed-borne *M. nivale* both pre- and post-emergence seedling symptoms were significantly reduced by the addition of fuberidazole (MBC). Tebuconazole, a similar compound to triadimenol, was again considered by Wainwright and Link (1987) to require the addition of another active ingredient, possibly fuberidazole, to increase *M. nivale* seedling blight control.

There is now evidence of *M. nivale* isolates which are resistant to MBC fungicides. In the U.K. Locke *et al.* (1987) tested five hundred and eighty seven isolates of *M. nivale* and found 92.1% to be resistant to benomyl. After twelve years'

use, reduced control from Baytan (triadimenol + fuberidazole, Bayer plc. Bury St Edmunds, Suffolk, U.K.) was observed in areas of Northern Germany (Scheinflug and Duben, 1988). Scheinflug and Duben concluded that in a seed treatment an MBC should not be used as the only component which is effective against *M. nivale*. They suggested that the most successful method of control would be a mixture of active ingredients against which there is no cross-resistance. The existence of *M. nivale* isolates resistant to MBC's was confirmed in an extensive U.K. survey of wheat stem-base isolates of *M. nivale* by Pettitt *et al.* (1993) and wheat seed isolates by Parry *et al.* (1995).

Compounds from other chemical groups such as fenpiclonil and fludioxonil (phenylpyrroles) (Koch and Leadbeater, 1992); guazatine (guanadine) (Jackson *et al.*, 1973) and bitertanol (DMI) (Morris *et al.*, 1994) have also been shown to have activity against *Fusarium* seedling blight although the effectiveness of the fungicide carboxin (carboxamide) seems unclear. Uoti (1979) used carboxin on artificially inoculated seed under glasshouse conditions and observed little effect on disease from the seed treatment. Subsequently Klein and Burgess (1987) showed that carboxin used alone at commercial rates had no effect on seedling blight from seed naturally infected with *F. graminearum* and Scheinflug and Duben (1988) showed that carboxin had insufficient action for the control of *M. nivale*. However, according to Noon and Jackson (1992) a 334% increase in emergence was observed in the field over non-treated seed when winter wheat seed with 72% *M. nivale* infection was treated with carboxin + thiabendazole. Carboxin is currently used in Europe in

mixture with thiram and its effectiveness against *Fusarium* seedling blight was demonstrated by Mihuta-Grimm and Forster (1989) and Jackson *et al.* (1994).

CHAPTER 2

General materials and methods

MATERIALS AND METHODS

Pathogens used in Experiments

Table 2 Pathogens used in the experiments and the origin of isolates

Pathogen	Host
<i>Microdochium nivale</i>	winter wheat seed (Table 3)
<i>Fusarium culmorum</i>	winter wheat seed (Table 3) winter wheat stem-base

Host Cultivars

Winter wheat (*Triticum aestivum* L.) Cultivars: Admiral, Avalon, Beaver, Brigadier, Estica, Haven, Hunter, Lynx, Mercia, Riband and Slejpner.

Culture of Host

(i) Seed surface sterilization

Seeds were placed in a solution of sodium hypochlorite (1% available chlorine) for three minutes. Seeds were then washed three times in sterile distilled

water placed on sterile blotting paper and then dried in a flow of sterile air.

(ii) Seedling growth media

Wheat seeds were planted into a sandy loam soil-based compost. John Innes seed and John Innes No.2 (J. Arthur Bower's, Firth Road, Lincoln, U.K.) compost was used. Before planting the compost was passed through a 5 mm sieve and then sterilized at 121°C for 60 minutes three times with 24 hours between sterilizations.

(iii) Planting of seed

Seeds were planted at a depth of 2 cm. Details of the containers, soil and soil quantities used together with the number of seeds planted per container are given in Table 4.

Culture of Pathogen

(i) Aseptic techniques

Glassware, pathogen growth media, distilled water and other autoclavable equipment was sterilized by heat treatment at 121°C for 20 minutes in an autoclave. Aseptic operations were performed in a laminar flow cabinet following the sterilization of all surfaces with alcohol.

Table 3 Cultivar, pathogen, source and harvest year of naturally contaminated winter wheat seed used during the work

No.	cultivar	pathogen	source	harvest year
1	Admiral	<i>M. nivale</i>	Buckie, Grampian, Scotland	1992
2	Avalon	<i>M. nivale</i>	Eriswell, Suffolk, England	1993
3	Beaver	<i>M. nivale</i>	Boarders, Scotland	1994
4	Brigadier	None	Docking, Norfolk, England	1994
5	Estica	<i>M. nivale</i>	Haverhill, Suffolk, England	1993
6	Haven	<i>M. nivale</i>	Boarders, Scotland	1993
7	Haven	<i>F. culmorum</i>	Edgmond, Shropshire, England	1994
8	Haven	<i>F. culmorum</i>	Edgmond, Shropshire, England	1994
9	Haven	<i>F. culmorum</i>	Edgmond, Shropshire, England	1994
10	Haven	None	Edgmond, Shropshire, England	1994
11	Hunter	<i>M. nivale</i>	Scotland	1994
12	Hunter	<i>M. nivale</i>	Owlesbury, Hampshire, England	1993
13	Lynx	<i>M. nivale</i>	Stowmarket, Suffolk, England	1993
14	Mercia	None	Cambridge, Cambridgeshire, England	1993
15	Riband	<i>M. nivale</i>	Scunthorpe, Humberside, England	1993
16	Riband	<i>M. nivale</i>	Invergowrie, Tayside, Scotland	1993
17	Riband	<i>M. nivale</i>	Driffield, North Yorkshire, England	1993
18	Riband	<i>M. nivale</i>	Scotland	1993
19	Riband	<i>M. nivale</i>	Eriswell, Suffolk, England	1993
20	Riband	<i>M. nivale</i>	Yorkshire, England	1993
21	Riband	<i>M. nivale</i>	Boarders, Scotland	1993
22	Riband	None	Unknown	1994
23	Slejpner	<i>M. nivale</i>	Eriswell, Suffolk, England	1993

(ii) Storage of pathogen isolates

Single spore isolates of *M. nivale* and *F. culmorum* were produced from cultures of the pathogens isolated from plant material. Sporodochia were removed from cultures grown on potato dextrose agar (PDA) and the spores suspended in sterile distilled water. A 100 µl sample of the spore suspension was spread over tap water agar (TWA) in a 9 cm Petri dish and incubated at 15°C. After 24 hours single germinated conidia were located using a low powered microscope (x40) and transferred to separate Petri dishes containing 15 ml of sucrose nutrient agar (SNA). The Petri dishes were then sealed with "Parafilm" (American National Can, Greenwich, U.S.A.) and incubated at 15° for 10-14 days, after which they were transferred to a refrigerator and stored at 4°C.

(iii) Production of spores

Microdochium nivale and *F. culmorum* macroconidia were produced from colonies grown on PDA. Sub-cultures of the fungus were produced by transferring a 5 mm plug of inoculum from the edge of an actively growing stock culture, using a sterile cork borer, to a Petri dish containing 15 ml of PDA. Sub-cultures were then incubated at 15°C in darkness for 10-14 days. To induce sporulation sub-cultures were subjected to specific light regimes for a further 10 days. Cultures of *F. culmorum* were illuminated by Philips 365 nm black light fluorescent tubes on a 12 hour photoperiod at 20°C. Cultures of *M. nivale* were illuminated by Philips cool-white fluorescent tubes on a twelve hour photoperiod at 15°C.

Table 4 Details of the containers used and their dimensions, the types of compost used and the seed planting rates.

Container	Dimensions (mm)	compost used	Seeds per container
Plastic tray - ½ seed tray (Ward, Wednesbury, West Midlands, U.K.)	215x155x45	J. I. No. 2 (950 g)	35
Plastic tray - ¼ seed tray (Ward, Wednesbury, West Midlands, U.K.)	165x95x45	J. I. No. 2 (480 g)	20
Plastic pot - Optipot 7M	70x70x80	J. I. seed (230 g)	16
Glass jar - clear glass, wide screw neck 250 ml (Fisons, Bishop Meadow lane, Loughborough, Leicestershire, U.K.)	60x60x120	J. I. seed (60 g)	10

(iv) *Preparation of spore suspensions*

Macroconidia were dislodged and washed from the surface of sporulating cultures using a sterile spatula and 5 ml of sterile distilled water. The resultant spore suspension was then passed through three layers of muslin to remove hyphal fragments and then centrifuged at 5000 r.p.m. for 10 minutes. Following centrifugation, the supernatant was decanted and the spores suspended in sterile distilled water. The concentration of spores was determined using a haemocytometer (Improved Neubauer, Webber Scientific International Ltd, 40 Udney Park Road,

Teddington, Middlesex, U.K.) and then adjusted to the required concentration.

Inoculation of Seed

Surface sterilized seeds were inoculated with spores at the rate of 10^4 *F. culmorum* spores and 10^5 *M. nivale* spores per 25 g seed. The seed and spore suspension were mixed together in a 250 ml conical flask and shaken by hand for three minutes.

Isolation from Plant Material

(i) Seed

Four hundred surface sterilized seeds were transferred aseptically onto PDA supplemented with the antibiotics streptomycin sulphate ($100\ \mu\text{g ml}^{-1}$), neomycin sulphate ($50\ \mu\text{g ml}^{-1}$) and chloramphenicol ($50\ \mu\text{g ml}^{-1}$) to exclude bacterial contaminants. Five seeds were placed on each Petri dish. The Petri dishes were then sealed with "Parafilm" and placed in an unilluminated incubator at 15°C . After 10-14 days the resultant fungal colonies were examined and identified.

(ii) Seedling stem-bases

The bottom 20 mm of the stem-base was cut transversely from each seedling using a sterile scalpel. Excised stem-base segments were then surface sterilized in a

solution of sodium hypochlorite (1% available chlorine) for three minutes and then washed three times in sterile distilled water. Surface sterilised stem segments were then transferred aseptically onto sterile filter paper to dry in a flow of sterile air before placing onto PDA. Dry stem-base segments were placed five to a 9 cm Petri dish onto PDA supplemented with the antibiotics streptomycin sulphate ($100\ \mu\text{g ml}^{-1}$), neomycin sulphate ($50\ \mu\text{g ml}^{-1}$) and chloramphenicol ($50\ \mu\text{g ml}^{-1}$) to exclude bacterial contaminants. The Petri dishes were sealed with "Parafilm" and then placed in an unilluminated incubator at $15\ ^\circ\text{C}$. After 10-14 days the resultant fungal colonies were examined and identified.

(iii) Wheat plant stem-bases

Plants were washed under running water to remove soil particles. Cylindrical segments 40 mm in length, which included the lowest node, were cut transversely from the stem-base of each main tiller using a sterile scalpel. Excised stem sections were then surface sterilized in a solution of sodium hypochlorite (1% available chlorine) for three minutes and then washed three times in sterile distilled water. Surface sterilised sections were then transferred aseptically onto sterile filter paper to dry in a flow of sterile air. When dry, each stem-base section was cut longitudinally in half using a sterile scalpel. One half of the stem-base section was placed onto 15 ml of PDA in a Petri dish and the other onto 15 ml of PDA amended with benomyl (Benlate $500\ \text{g kg}^{-1}$ benomyl, Du Pont U.K. Ltd, Agricultural Products Dept, Wedgwood Way, Stevenage, Hertfordshire, U.K.) ($10\ \mu\text{g ml}^{-1}$). Both media

were supplemented with the antibiotics streptomycin sulphate ($100\ \mu\text{g ml}^{-1}$), neomycin sulphate ($50\ \mu\text{g ml}^{-1}$) and chloramphenicol ($50\ \mu\text{g ml}^{-1}$) to exclude bacterial contaminants. The Petri dishes were sealed with "Parafilm" and then placed in an unilluminated incubator at $15\ ^\circ\text{C}$. After 10-14 days the resultant fungal colonies were examined and identified.

Identification of Isolates

(i) Colony characteristics and spore morphology

In most cases *M. nivale* and *F. culmorum* isolates could be identified from their colony characteristics and spore morphology when grown on PDA. However, isolates which proved difficult to identify were grown on PSA and SNA. After 10-14 days at 20°C with a 12 hour light/dark regime colonies on PSA plates had developed a characteristic morphology and pigmentation, and cultures on SNA plates produced uniform macroconidia. Cultures were then identified from colony characteristics and microscopic examination of conidia and conidiogenous cells.

(ii) Molecular classification of *M. nivale* isolates into the sub-groups *M. nivale* var. *nivale* and *M. nivale* var. *majus* using a random amplified polymorphic DNA (RAPD) assay

Classification of *M. nivale* isolates into the sub-groups *M. nivale* var. *nivale* and *M. nivale* var. *majus* using a random amplified polymorphic DNA (RAPD) assay

(Lees *et al.*, 1995) was performed by D. W. Parry and H. N. Rezanoor and details of the technique used can be found in Parry *et al.* (1995).

Assessment of the Rate of Seedling Emergence

The wheat seedlings that emerged were counted daily. Final emergence was assessed 24 days after planting when no further seedlings had emerged on five successive days. The mean seedling emergence time in days was calculated using Equation 1 (Khah *et al.*, 1986). Where n is the number of seedlings which emerged on day D , and D is the number of days from planting. The mean rate of emergence was calculated as the reciprocal of the mean seedling emergence time in days .

$$\text{mean seedling emergence time (days)} = \frac{(D \times n)}{n} \quad \text{Equation 1}$$

Disease Assessment

The severity of seedling disease was assessed in three ways. The first and most severe symptom was pre-emergence death (non-emergence). The second most severe symptom was post-emergence death. The third and least severe symptom of disease was an assessment of necrosis on emerged seedlings. The seedlings were assigned one of five disease values (0, 1, 2, 3 or 4) according to the severity of the symptoms

present (Table 5) and an overall disease score was then calculated. The sum of the individual seedling disease values was divided by the product of maximum disease value (4) and the number of seedlings assessed, and then multiplied by 100. The minimum and maximum disease scores possible were 0 and 100 respectively.

Table 5 Key used to assess the severity of *Fusarium* seedling blight symptoms caused by *Microdochium nivale*, on wheat seedlings.

Disease symptoms	Value
None	0
1-2 lesions on the coleoptile	1
More than 2 separate lesions on the coleoptile	2
Total necrosis of the coleoptile	3
Total necrosis of the coleoptile and deformed seedling growth	4

Soil Water

(i) Determination of soil water content

The water content of the soil was determined by oven drying (Anon., 1977a). Five 30 g sub-samples of compost were oven dried at 102°C to constant weight. Each sub-sample was then reweighed and the percentage soil water content by mass was calculated from the difference in weights before and after drying and presented as a

percentage of the mass of dry soil (equation 2).

$$\frac{\text{Weight of wet soil} - \text{weight of dry soil}}{\text{Weight of dry soil}} \times 100 = \text{Soil water content by mass (\%)} \quad \text{Equation 2}$$

(ii) Determination of soil water retention curve

Soil water retention curves relating soil water content by mass to soil matric potential for John Innes seed and John Innes No. 2 compost were obtained by the pressure membrane method (Anon., 1977b). A 1 kg sample of soil was passed through a 5 mm sieve, saturated with water and left to stand for two days. Sub-samples (50 g), of the water saturated soil, were placed in each of twelve separate pressure cells. The cells were then sealed and allowed to drain under applied pressure. The pressures used were; 0.06 MPa, 0.08 MPa, 0.10 MPa, 0.15 MPa, 0.20 MPa, 0.30 MPa. Two replicate cells were used at each pressure. These constant pressures were applied to the cells for 10 days to allow the system to reach equilibrium. When at equilibrium, each cell was depressurised and the soil divided into two further sub-samples.

The percentage soil water content by mass was then calculated using equation 2. The mass of water was then plotted against the logarithm of the applied pressure. The line of best fit gave the soil water retention curve for the soil.

CHAPTER 3

**Comparison of methods for the detection of *Microdochium nivale*
contamination of winter wheat seed.**

INTRODUCTION

Seed-borne inoculum of *M. nivale* can cause seedling blight if contaminated seeds are sown in conditions conducive to disease development. Hewett (1983) sowed seed lots of winter wheat, naturally contaminated by *M. nivale*, in the field and observed differences in emergence from the seed lots used. He associated the low emergence from some seed lots to the high incidence of *M. nivale* detected in the seed by agar plating (Hewett, 1965) prior to sowing. He also associated severe winter kill of seedlings with the more heavily contaminated seed lots. The examination of wheat seed for *M. nivale* contamination prior to sowing can therefore play an important role in assessing the likelihood of seedling blight symptoms in the subsequent crop.

The degree of *M. nivale* contamination of a batch can be assessed using various methods, each of which assesses the percentage of seeds that is contaminated by *M. nivale*. In official seed testing stations, seed lots are usually examined for *M. nivale* contamination following a period of incubation. Three types of media may be used; paper, often paper towels, sand or artificial composts or agar (Anon., 1985). The paper towel method provides a relatively quick and inexpensive test requiring little incubator space. Tests in sand or compost require more space, but are useful when seed has been treated with chemicals which may cause phytotoxicity. In sand or compost the risk of phytotoxicity is reduced as the chemical seed treatment is able to diffuse more readily away from the seed into the medium than in the paper towel test. The subsequent estimation of *M. nivale* contamination using these two methods

is based in part on the development of disease symptoms in seedlings following incubation. If seedlings grow abnormally, or lesions develop, then they are deemed to have come from a contaminated seed.

When the agar method is used, the estimation of *M. nivale* contamination is based on the recovery and growth of the pathogen from seeds placed on an agar medium. Therefore, unlike the paper and sand methods where seed contamination is based on the expression of disease symptoms, with the agar method contamination is based on the recovery of the pathogen from individual seeds.

Seed contamination based on the visual assessment of wheat grains is possible for some pathogens. For example, "blackpoint", caused by *Alternaria* spp. is assessed in a sample by recording the percentage of grains showing discolouration. In addition, Grabarkiewicz-Szczesna and Chelkowski (1993) reported a relationship between the quantity of metabolites produced by *Alternaria* spp. in wheat grains and the incidence of seeds with "blackpoint". A similar relationship between metabolite production by *Fusarium* spp. (deoxynivalenol) and the occurrence of tombstone grains in Canadian wheat seed was established by Shotwell *et al.* (1985) and Clear and Patrick (1990). The contamination of wheat grains by *Fusarium* spp., most notably *F. culmorum*, which was associated with a pink discolouration and shrivelling of Polish grain (Perkowski and Chelkowski, 1991) and *F. graminearum* which was associated with shrivelled wheat grain with a light colour in Kansas and Nebraska, U.S.A. (Bechtel *et al.*, 1985). However, wheat grains contaminated by *Fusarium* spp. can occur frequently without any visual symptoms (Wakulinski and Chelkowski, 1993) and

wheat grains contaminated by *M. nivale* usually show no visual symptoms of contamination (Chelkowski, personal communication.).

The aim of the following work was fourfold. (i) To compare the paper towel and agar methods with respect to the estimation of *M. nivale* seed contamination. (ii) To evaluate the effects of seed surface sterilization with sodium hypochlorite and media incorporation of the fungicide benomyl on the results of the agar method. (iii) To establish if there is a relationship between *M. nivale* contamination of wheat seed and the shrivelling or discolouration of individual grains. (iv) To evaluate the suitability of seed samples for use in subsequent experiments.

MATERIALS AND METHODS

(i) Comparison of the Paper Towel and Agar Methods for the Estimation of *M. nivale* Contamination of Winter Wheat Seed

Fourteen seed lots of wheat seed naturally contaminated by *M. nivale* were used in this study. The seed lots used were Nos 1, 2, 3, 6, 11, 12, 13, 15, 17, 18, 19, 20, 21, and 23 (Table 3). Details are given in Table 6.

The paper towel method

For each of the 14 seed lots tested, four replicates of 100 seeds were placed

evenly on three layers of separate paper towels which had been pre-moistened with sterile distilled water. Each towel was then rolled to form a cylinder and secured top and bottom with rubber bands. The paper towel rolls were then placed individually into plastic bags, sealed with rubber bands and incubated at 20°C. Seedlings were assessed for abnormal growth, according to the International Seed Testing Association regulations for wheat seedling growth defects (Anon., 1985), and the development of seedling blight symptoms after 14 days.

The agar method

Four hundred seeds from each of the 14 seed lots tested were treated with sodium hypochlorite (NaOCl) (see Chapter 2) and then placed crease down onto potato dextrose agar (PDA) supplemented with the antibiotics streptomycin sulphate (100 µg ml⁻¹), neomycin sulphate (50 µg ml⁻¹) and chloramphenicol (50 µg ml⁻¹) (see Chapter 2), five to a Petri dish. The resultant fungal colonies were identified on the basis of their colony and spore morphology after incubation for 14 days at 15°C.

Data analysis

Data were analysed using the Mann-Whitney U test. Statistical analysis was performed on a personal computer using the software program Statgraphics (Manugistics, Maryland, U.S.A.).

Table 6 Code number and cultivar of the batches of winter wheat seed used.

code	cultivar	code	cultivar
1	Admiral	15	Riband
2	Avalon	17	Riband
3	Beaver	18	Riband
6	Haven	19	Riband
11	Hunter	20	Riband
12	Hunter	23	Slejpner
13	Lynx	21	Riband

(ii) The Effect of Seed Surface Sterilization and The Incorporation of Benomyl into Potato Dextrose Agar on the Recovery of *M. nivale* from Wheat Seed.

Six (13, 15, 17, 18, 19 and 20 (Table 3)) of the 14 seed lots were used to investigate the effects that seed surface sterilization and the incorporation of benomyl into potato dextrose agar had on the recovery of *M. nivale* from seed.

Four treatments were used (Table 7). One hundred and sixty seeds from each seed lot were treated with sodium hypochlorite (NaOCl) 1% available chlorine for three minutes (see Chapter 2). Eighty of the seeds were placed crease down five to a Petri dish onto PDA supplemented with the antibiotics streptomycin sulphate (100 $\mu\text{g ml}^{-1}$), neomycin sulphate (50 $\mu\text{g ml}^{-1}$) and chloramphenicol (50 $\mu\text{g ml}^{-1}$) (Treatment

1), and the remaining 80 onto PDA supplemented with the antibiotics streptomycin sulphate ($100\ \mu\text{g ml}^{-1}$), neomycin sulphate ($50\ \mu\text{g ml}^{-1}$) and chloramphenicol ($50\ \mu\text{g ml}^{-1}$) and amended with benomyl ($10\ \text{mg/l}$) (Pettitt *et al.*, 1993) (Treatment 3). A further 160 seeds from each seed lot were treated with sterile distilled water. Eighty of the seeds were then placed crease down five to a Petri dish onto PDA (Treatment 2) and the remaining 80 onto PDA amended with benomyl ($10\ \text{mg/l}$). Again the PDA was supplemented with antibiotics as previously described.

Data analysis

Data were analysed using analysis of variance. Statistical analysis was performed on a personal computer using the software program Statgraphics.

Table 7. List of treatments used to investigate the effects of seed surface sterilization by sodium hypochlorite (NaOCl) and potato dextrose agar (PDA) amended with benomyl on the recovery of *Microdochium nivale* from winter wheat seed.

Number	Treatment
1	NaOCl pre-treatment and benomyl-amended PDA
2	H_2O pre-treatment and benomyl-amended PDA
3	NaOCl pre-treatment and PDA
4	H_2O pre-treatment and PDA

(iii) The Relationship between *M. nivale* Contamination and the Appearance of Wheat Seeds

Three of the 14 seed lots were used to investigate the relationship between shrivelled and discoloured seed and contamination by *M. nivale*. The seed lots used were numbers 15, 18 and 19 (Table 6). Seven hundred seeds from each of the three seed lots were individually examined for shrivelling and discolouration and assigned to one of four categories (Table 8). *Microdochium nivale* contamination of individual seeds was then established following incubation on agar (see above).

Data analysis

Data were analysed using contingency tables and the chi squared test. Statistical analysis was performed manually with the aid of a pocket calculator.

Table 8. List of categories to which individual seeds were assigned following visual examination

Category	Seed features
1	Non-shrivelled seed with no discolouration
2	Non-shrivelled seed with discolouration
3	Shrivelled seed with no discolouration
4	Shrivelled seed with discolouration

RESULTS

(i) A Comparison of the Paper Towel and Agar Methods for the Estimation of *M. nivale* Contamination of Winter Wheat Seed

For the seed lots tested the percentage of seeds contaminated by *M. nivale* ranged from 3.5% to 72% as estimated by the paper towel method and from 4% to 72% as estimated by the agar method (Figure 2). The results from the two methods for an individual seed lot were often dissimilar. However, differences between the two methods were generally small and statistical analysis of the data showed them not to be significant ($P = 0.818$).

(ii) The Effect of Seed Surface Sterilization and the Incorporation of Benomyl into Potato Dextrose Agar on the Recovery of *M. nivale* from Wheat Seed

Differences in the recovery of *M. nivale* from seed following sterilization and the incorporation of benomyl into PDA were observed (Table 9). The data were analysed using factorial analysis of variance, with seed lot, NaOCl treatment and the addition of benomyl to agar as factors. Analysis showed that there were significant differences between the seed lots used ($P < 0.001$), NaOCl treatment ($P = 0.029$) and the amendment of agar with benomyl ($P = 0.008$) with respect to the recovery of *M.*

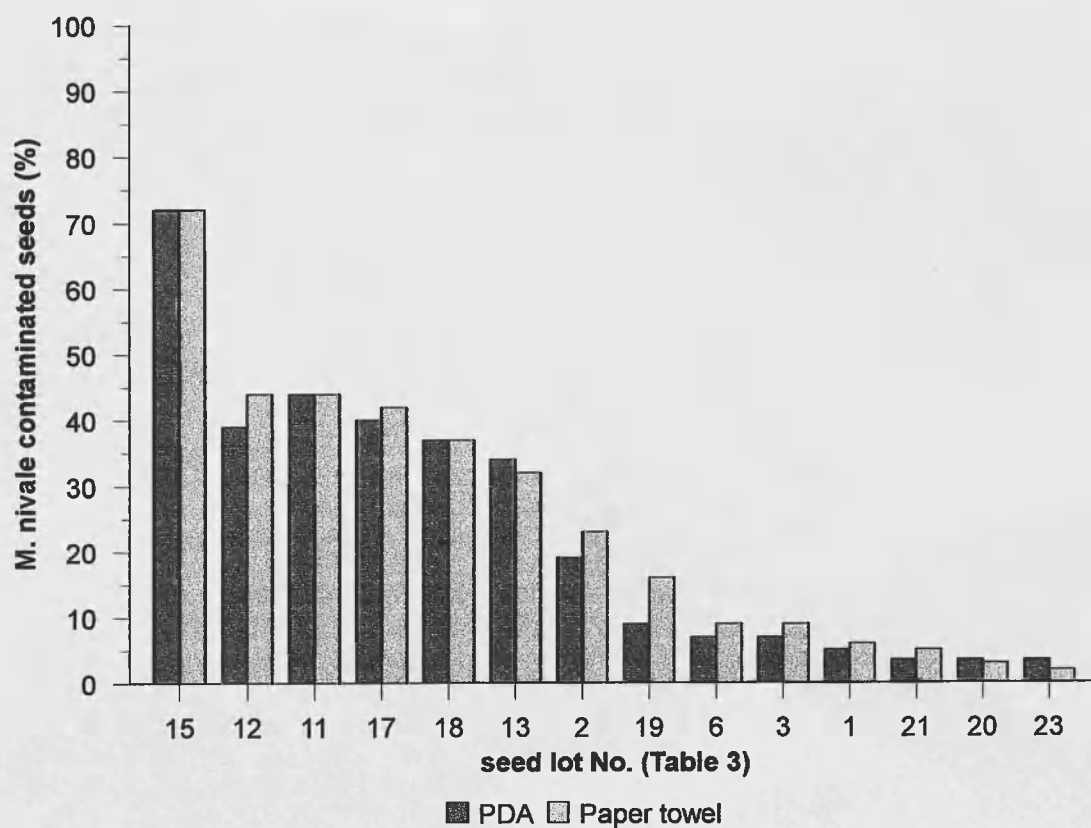


Figure 2 The percentage of *Microdochium nivale* contaminated seed in 14 lots of wheat seed (Table 3) as estimated by the paper towel and agar methods. Seed lots are ranked according to *M. nivale* contamination as estimated by the paper towel test.

nivale. However, there was a significant interaction ($P = 0.008$) between these three factors indicating that the effects of NaOCl pre-treatment and the use of benomyl amended agar were different with respect to each seed lot tested. For this reason, only individual treatment means are given in Table 9. The isolation of *M. nivale* isolates from seed lots 17 and 20 was unaffected by the treatments used ($P = 0.05$). However, differences were seen for the remaining seed lots. Pre-treatment with NaOCl and the use of benomyl amended agar significantly reduced ($P = 0.05$) the recovery of *M. nivale* compared with no NaOCl pre-treatment and the use of PDA and PDA amended with benomyl with seed lots 18 and 19 respectively. No NaOCl pre-treatment and PDA without benomyl significantly reduced ($P = 0.05$) the recovery of *M. nivale* from seed lot 15 compared to the other three treatments. In contrast pre-treatment with NaOCl significantly reduced ($P = 0.05$) the recovery of *M. nivale* when PDA without benomyl was used.

(iii) The Relationship between *M. nivale* Contamination and The Appearance of Wheat Seeds

The percentage of *M. nivale* contaminated seeds in each appearance category is given in Table 10. Overall, there was no obvious association between the seed appearance characteristics examined and *M. nivale* seed contamination. *Microdochium nivale* was recovered from both shrivelled and non-shrivelled seeds

and from seeds with and without discolouration. However, there were differences between the seed lots with respect to the percentage of *M. nivale*-contaminated seeds within each appearance category. The greatest contamination (75%) was seen from shrivelled discoloured seed from seed lot No. 15 and the least (0%) from shrivelled discoloured seed from seed lot No. 19.

Table 10 The percentage of *Microdochium nivale* contaminated seed in each of the seed appearance categories for seed lots 15, 18, and 19 (Table 3).

Seed lot No.	Shrivelled seed (%)		Non-shrivelled seed (%)	
	Discolouration	no discolouration	Discolouration	no discolouration
15	75.0	55.4	66.9	62.1
18	25.0	25.0	28.8	23.2
19	0.0	23.1	50.0	19.9

Table 9 Mean number of *Microdochium nivale* isolates recovered per Petri dish from winter wheat seed on potato dextrose agar (PDA) or PDA amended with benomyl (10 mg/l) following pre-treatment with sodium hypochlorite (NaOCl) or water (Table 2).

Seed lot (No.)	Treatment			
	+ NaOCl,	no NaOCl,	+ NaOCl,	no NaOCl,
	+ benomyl	+ benomyl	no benomyl	no benomyl
13	1.63	1.94	1.12	2.56
15	3.25	3.19	2.56	0.88
17	2.00	2.19	1.62	1.88
18	1.62	2.00	1.44	2.31
19	0.69	1.44	1.00	1.31
20	0.18	0.18	0.06	0.12
SEM = 0.262 D of F = 360 CV = 67%				

DISCUSSION

The paper towel and agar methods were not found to be significantly different with respect to their estimates of the percentage *M. nivale* contamination of the seed lots tested. This suggests that either of the assessment criteria used ie. disease symptoms or abnormal seedling growth or the recovery of the pathogen from seed gives a representative estimate of *M. nivale* contamination. However, differences

between the estimates of contamination for a given seed lot were seen with different NaOCl and benomyl treatments when seeds were placed on PDA.

A sodium hypochlorite pre-treatment (0.5% for 10 minutes) was found by Limonard (1968) to increase the recovery of *M. nivale* from wheat seeds. In two separate experiments he found that NaOCl pre-treatment increased the percentage of *M. nivale* recovered on PDA from 29% and 26% to 30% and 29% respectively, and from 12.8% and 26.0% to 15.5% and 34.3% respectively. He attributed the increase in the recovery of *M. nivale* following pre-treatment to the removal of other fungi. He suggested that the removal of pathogens like the *Fusarium* spp., *Septoria nodorum* and the saprophytes *Alternaria* spp. and *Epicoccum nigrum* allowed the slow growing and usually masked *M. nivale* to be identified. The increased isolation of *M. nivale* from wheat seed following NaOCl pre-treatment was also observed by De Tempe and Limonard (1973). They examined 137 samples of wheat seed following immersion in NaOCl (0.5%) for 10 minutes and then incubation on PDA. On average the recovery of *M. nivale* increased following pre-treatment from 12.8% to 16.2%, although they did not say if this increase was observed in each of the 137 seed samples. In the present study there was little evidence to suggest that treatment with NaOCl increased the recovery of *M. nivale* from seed. However, the seed lots selected for use in this study were largely free from fungal contaminants other than *M. nivale*, and contained other pathogens at only trace levels.

Limonard (1968) also highlighted the role of bacteria in the unsuccessful recovery of *M. nivale* from seeds on PDA. He found that certain bacteria out

competed *M. nivale* and that the addition of terramycin (100 µg / kg) stopped bacterial growth allowing increased recovery of *M. nivale*. The influence of bacteria was not examined in this study owing to the addition of antibiotics to the PDA. However, treatment with NaOCl could have had a significant effect on bacterial contamination.

As well as the removal of other pathogens and saprophytes from the seeds' surface following a sterilization pre-treatment, Neergaard (1977) suggested that the sterilization pre-treatment would also remove superficial inoculum. The removal of superficial *M. nivale* on the surface of wheat seed was cited by Cariddi (1982) as the reason for the observed reduced recovery of *M. nivale* on PDA. The recovery of *M. nivale* was reduced from 47% to 32% following pre-treatment with NaOCl (4%) for 5 minutes.

Direct evidence for the reduction in superficial inoculum following surface sterilization was given by Bateman (1983). Bateman used phenyl mercury acetate (PMA) as a surface sterilant on two seed lots of wheat. The percentage of *M. nivale* recovered from the outer epidermis and inner pericarp and testa was significantly lower in one of the seed lots following treatment with 1.0 µg Hg / seed (PMA). A reduction in the recovery of superficial *M. nivale* following pre-treatment with NaOCl (1%) for 10 minutes was also observed by Cristani (1992). Following pre-treatment, the percentage of *M. nivale* recovered from the pericarp was reduced from 34% to 22%, the endosperm from 22% to 14%, and the embryo from 18% to 4%. The reduced recovery of *M. nivale* in the present study from seed lots 18 and 19 following

NaOCl pre-treatment and plating on PDA with benomyl may therefore be a combination of the reduction of seed surface inoculum and the inhibition of growth of benomyl sensitive isolates, although benomyl sensitive isolates should be rare (Pettitt *et al.*, 1993; Parry *et al.*, 1995).

The results from the present study are therefore both in agreement with and in contradiction to previous observations. It is clear that the successful recovery of *M. nivale* from wheat seed is complex and that the individual characteristics of a given seed lot will affect the result obtained for a given method used.

There is little evidence from previous studies that *M. nivale* causes visible symptoms of contamination on wheat seed. In the present study there was only evidence of visible symptoms of contamination in one of the seed lots (No. 19, Table 3) examined. No obvious trend was seen in the data, with shrivelled seed without discolouration and non-shrivelled seed with discolouration having a greater number of contaminated seed than expected. It is clear then that shrivelling or discolouration alone cannot be considered as diagnostic features with respect to *M. nivale* contamination.

CHAPTER 4

The relationship between wheat seed weight, contamination by

***Microdochium nivale* and seedling disease.**

INTRODUCTION

The effect of *Fusarium* ear blight (FEB) caused by *M. nivale* on the grain yield of winter wheat is not clear. There are two components of yield that could be affected by *M. nivale* ear infection; the number of seeds per ear and the weight of individual seeds. Cassini (1981), in a review of *Fusarium* diseases of cereals in Western Europe, proposed that reduced yield following *M. nivale* ear infection was caused solely by a reduction in thousand grain weight (TGW). However, Cassini did not offer any evidence for this claim.

In contrast to *M. nivale*, the effect of *Fusarium* ear blight caused by *Fusarium* spp. on the yield of winter wheat is much clearer. A reduction in yield, associated with a decrease in the weight of individual wheat seeds was demonstrated by Snijders and Perkowski (1990) in field trials in the Netherlands following inoculation of wheat ears with *F. culmorum* conidia. The mean loss of yield of 9.7% was almost exclusively attributable to a reduction in seed weight. *Fusarium graminearum* has also been shown to affect wheat yield by reducing seed weight. Wong *et al.* (1992) observed that ear infection of wheat caused by *F. graminearum* in field plots in Canada reduced the thousand grain weight (TGW) of seed by 34%.

The reduced TGW observed following ear infections by *Fusarium* spp. has been attributed to a reduction in the weight of individual contaminated seeds following attack by the pathogen. Bechtel *et al.* (1985), working with wheat seed from the U.S.A., demonstrated that *F. graminearum* seed contamination, identified

by the presence of the mycotoxin deoxynivalenol (DON) and ergosterol, was most frequent in the lighter seeds. Work by Nakagawa and Yamaguchi (1989) showed that the majority of seeds naturally contaminated by what they described as *Fusarium roseum* were lighter than non-contaminated seeds in a sample of wheat in Japan. However, in contrast to this Perkowski and Chelkowski (1991), working in Poland, showed that the majority of *Fusarium* spp. contaminated wheat seeds, as identified by DON content, were the same size as non-contaminated seeds.

The aim of the work reported here was to determine the relationship between natural *M. nivale* seed contamination and the weight of individual wheat seeds and to investigate the relationship between *M. nivale* seed contamination, seed weight and the subsequent severity of *Fusarium* seedling blight symptoms.

MATERIALS AND METHODS

(i) The Relationship Between Natural *M. nivale* Seed Contamination, the Weight of Individual Wheat Seeds and Germination

The frequency distributions of seeds by weight for three seed lots of winter wheat seed cv. Riband (Nos 15, 18 and 19 (Table 3)), naturally contaminated by *M. nivale* were produced. Seven hundred seeds were taken at random from each seed lot, individually weighed and placed in one of eight weight categories; 10-20 mg, 21-30 mg, 31-40 mg, 41-50 mg, 51-60 mg, 61-70 mg, 71-80 mg and 81-90 mg.

The number of seeds contaminated by *M. nivale* was determined by isolating the pathogen from individual seeds. Seeds were placed in a solution of sodium hypochlorite (NaOCl) (1% available chlorine) for three minutes. Seeds were then washed three times in sterile distilled water, placed on sterile blotting paper and then dried in a flow of sterile air. The surface sterilized seeds were transferred aseptically onto PDA supplemented with the antibiotics streptomycin sulphate (100 $\mu\text{g ml}^{-1}$), neomycin sulphate (50 $\mu\text{g ml}^{-1}$) and chloramphenicol (50 $\mu\text{g ml}^{-1}$). Five seeds were placed on each Petri dish. The Petri dishes were then sealed with "Parafilm" and placed in an unilluminated incubator at 15°C. After 14 days the resultant fungal colonies were examined and identified on the basis of their colony characteristics. The number of seeds in each weight category producing *M. nivale* colonies was counted. This allowed the production of frequency distributions by seed weight for contaminated seeds.

The germination of winter wheat seeds on PDA was assessed after 14 days. Seeds were deemed to have germinated successfully if they had reached growth stage (GS) 09 (Zadoks *et al.*, 1974). The number of *M. nivale* contaminated and non-contaminated seeds which had germinated were counted and expressed as a percentage of the total in each weight category.

(ii) The Relationship between *M. nivale* Seed Contamination, Seed Weight and the Severity of *Fusarium* Seedling Blight and Subsequent Seedling Growth

Sixteen treatments were used in this experiment (Table 11). Each treatment contained either heavy or light seeds from one of four seed lots of winter wheat cv. Riband (Nos 15, 18, 19 naturally contaminated by *M. nivale* and 22 without *M. nivale* contamination (Table 3)). Half of the seed was treated with a fungicide seed treatment (guazatine), the other half was untreated.

From each of the four seed lots, 166 large seeds and 166 small seeds were selected at random. This produced eight seed samples. Seeds in each of the eight samples were then weighed individually and the mean seed weight for each sample was calculated (Table 11). Seeds from each sample were then surface sterilized in NaOCl as described previously. The seeds were then divided into two equal sub-samples of 83 seeds. Of the sixteen sub-samples produced, eight were treated with Rappor (50g l⁻¹ guazatine, DowElanco) at the rate of 200ml (100 kg)⁻¹ of seed by mixing the seed and seed treatment together in a 125ml glass screw cap bottle. The other eight sub-samples were left untreated.

Sixteen seeds from each treatment were planted to a depth of 2 cm onto 230g of sterile compost John Innes No.2 in plastic pots (70 x 70 x 80mm). Before planting the compost was passed through a 5 mm sieve and then sterilized at 121°C for 60 minutes three times with 24 hours between sterilizations. Five replicates of each treatment were used giving a total of 80 pots which were placed in a controlled

environment cabinet (Conviron, Controlled Environments Ltd, Winnipeg, Manitoba, Canada) according to a fully randomised block design. Environmental conditions were set to 8°C for 8 h of light and 6°C for 16 h of darkness and 75% relative humidity.

Assessments of seedling emergence, disease severity and seedling growth

The number of emerged seedlings per tray was counted daily until no further seedlings emerged on five successive days. The mean seedling emergence time in days was calculated using Equation 1 as previously described (Khah *et al.*, 1986) (Chapter 2). The mean rate of emergence was calculated as the reciprocal of the mean seedling emergence time in days.

The severity of disease on emerged seedlings was assessed visually 24 days after planting. The seedlings were assigned one of five disease values (0, 1, 2, 3, or 4) according to the severity of the symptoms present (Table 5). The sum of the individual seedling disease values was then divided by the number of seedlings assessed per pot to produce a mean disease score per seedling.

Following disease severity assessment, the length of coleoptile, shoot and roots of each seedling were measured. Seedlings were then oven dried at 102°C to constant weight, and their weight recorded.

Data analysis

Coleoptile length, shoot length, root length and mean symptom severity scores

were analysed statistically using analysis of variance (ANOVA). Analysis was performed on a personal computer using the software program Statgraphics.

Table 11 Treatment numbers, seed lot number, mean seed weight of winter wheat seed and fungicide treatment used in the experiment.

Treatment No.	Seed lot	Seed weight category	Mean Seed weight (mg)	Fungicide (guazatine)
1	15	heavy	5.87	yes
2	15	heavy	5.81	no
3	15	light	1.82	yes
4	15	light	1.88	no
5	19	heavy	4.99	yes
6	19	heavy	4.93	no
7	19	light	1.51	yes
8	19	light	1.50	no
9	18	heavy	5.58	yes
10	18	heavy	5.41	no
11	18	light	2.13	yes
12	18	light	2.17	no
13	22	heavy	4.95	yes
14	22	heavy	5.12	no
15	22	light	2.18	yes
16	22	light	2.01	no

RESULTS

(i) The Relationship between *M. nivale* Contamination, the Weight of Individual Wheat Seeds and the Germination of Seeds Grown on PDA

Frequency distributions of seeds by weight were generated for each of the seed lots tested (Figures 3, 4 and 5). The frequency distributions of *M. nivale* contaminated seeds were very similar on a seed lot basis to the distribution of seeds as a whole.

The germination of *M. nivale* contaminated seeds was poor (seed lot No. 15 = 22%, No. 19 = 24% and No. 18 = 21%). There was no clear relationship between contamination, seed weight and the germination of seed lot number 18 (Figure 8). The germination of light (10-20 mg) contaminated seeds from seed lots numbers 15 and 19 was lower, 0% and 25% respectively, than for heavy (71-80 mg) contaminated seeds, 36% and 100% respectively (Figures 6 and 7).

In contrast to *M. nivale* contaminated seeds, the germination of uncontaminated seeds was good (seed lot No. 15 = 94%, No. 19 = 96% and No. 18 = 99%) and was unaffected by seed weight (Figures 6, 7 and 8).

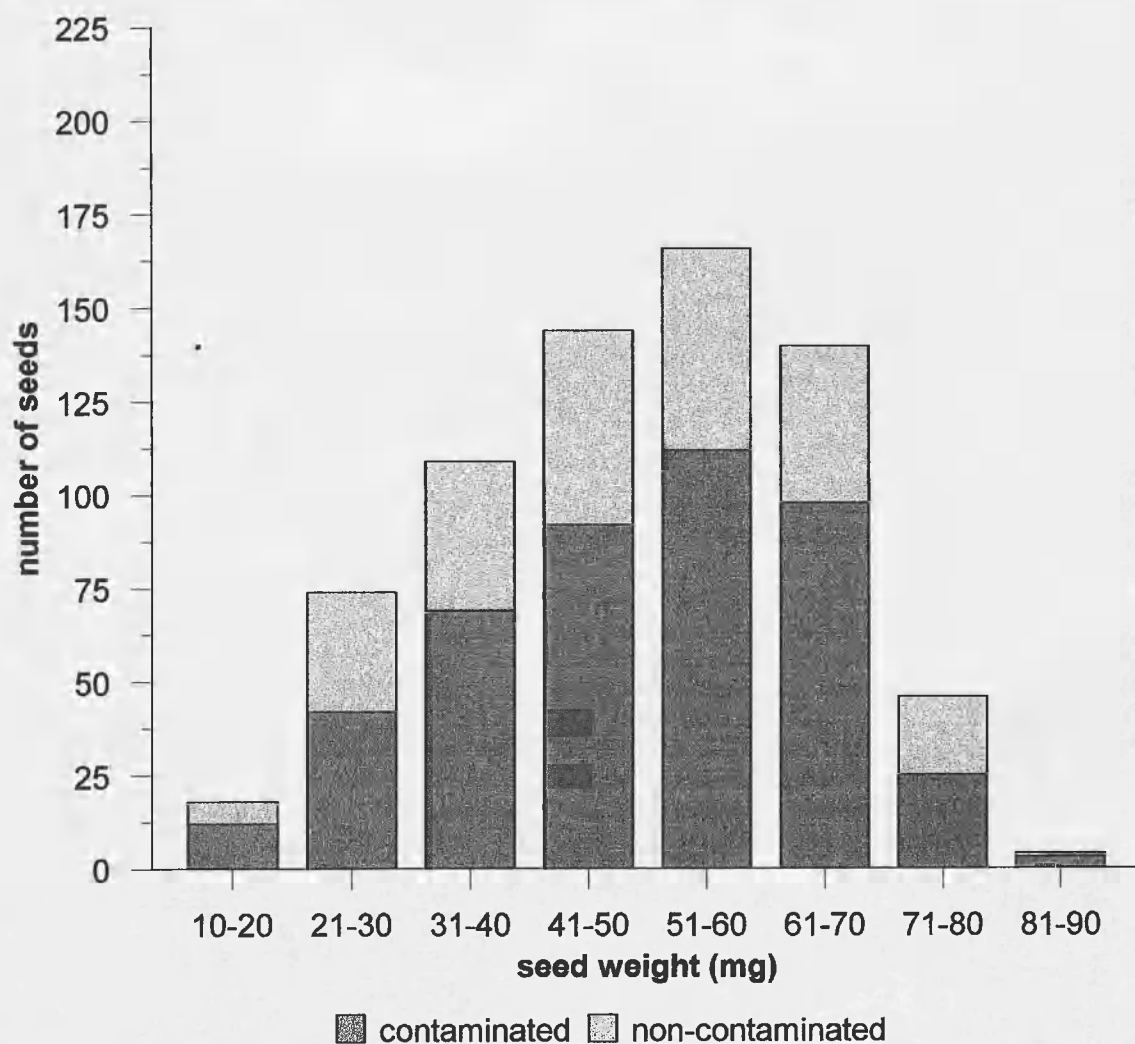


Figure 3 Frequency distribution by weight of wheat seed (lot No. 15, see Table 3). *Microdochium nivale* contaminated and non-contaminated seed in each weight category are presented.

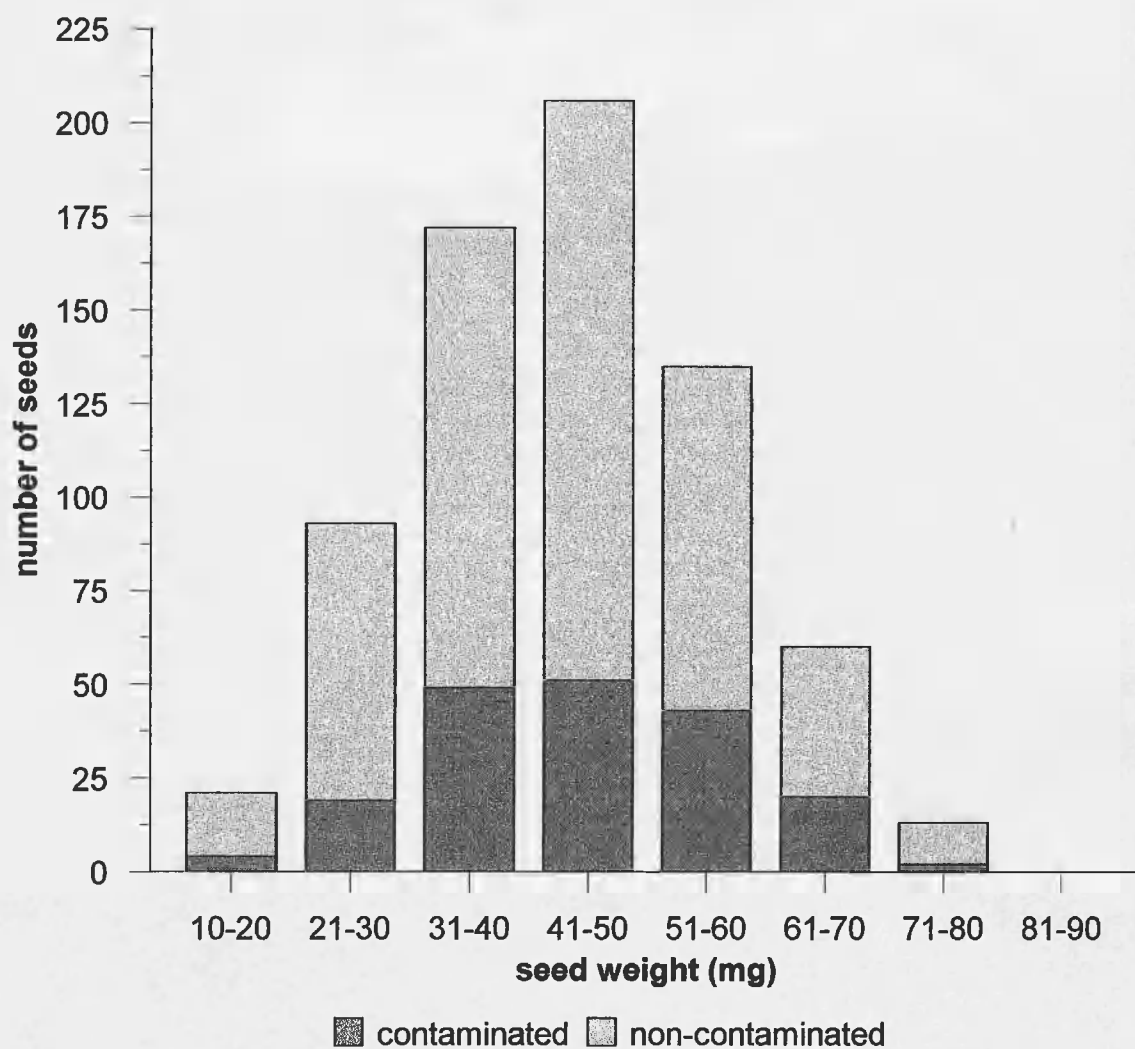


Figure 4 Frequency distribution by weight of wheat seed (lot No. 19, see Table 3). *Microdochium nivale* contaminated and non-contaminated seed in each weight category are presented.

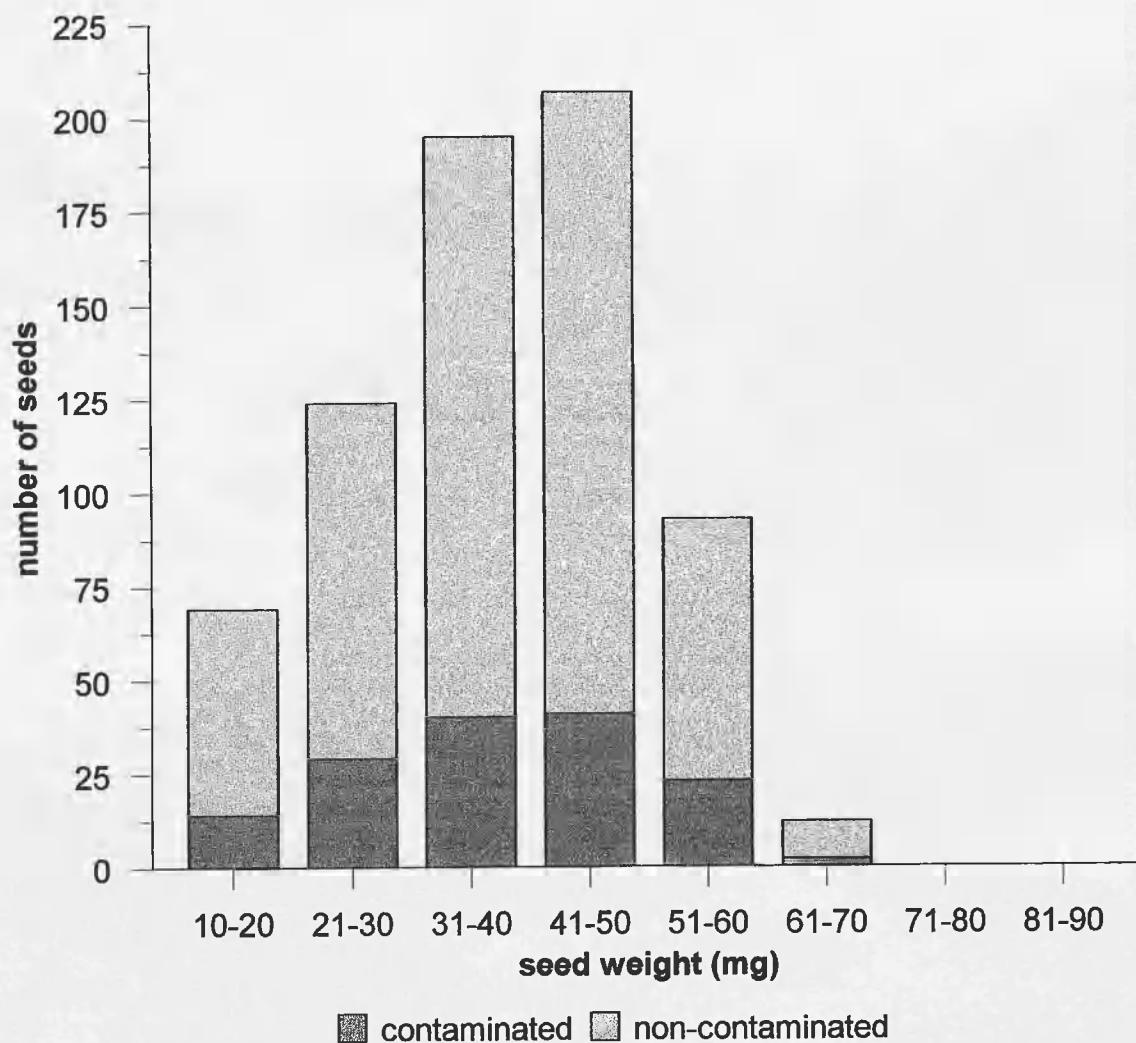


Figure 5 Frequency distribution by weight of wheat seed (lot No. 18, see Table 3). *Microdochium nivale* contaminated and non-contaminated seed in each weight category are presented.

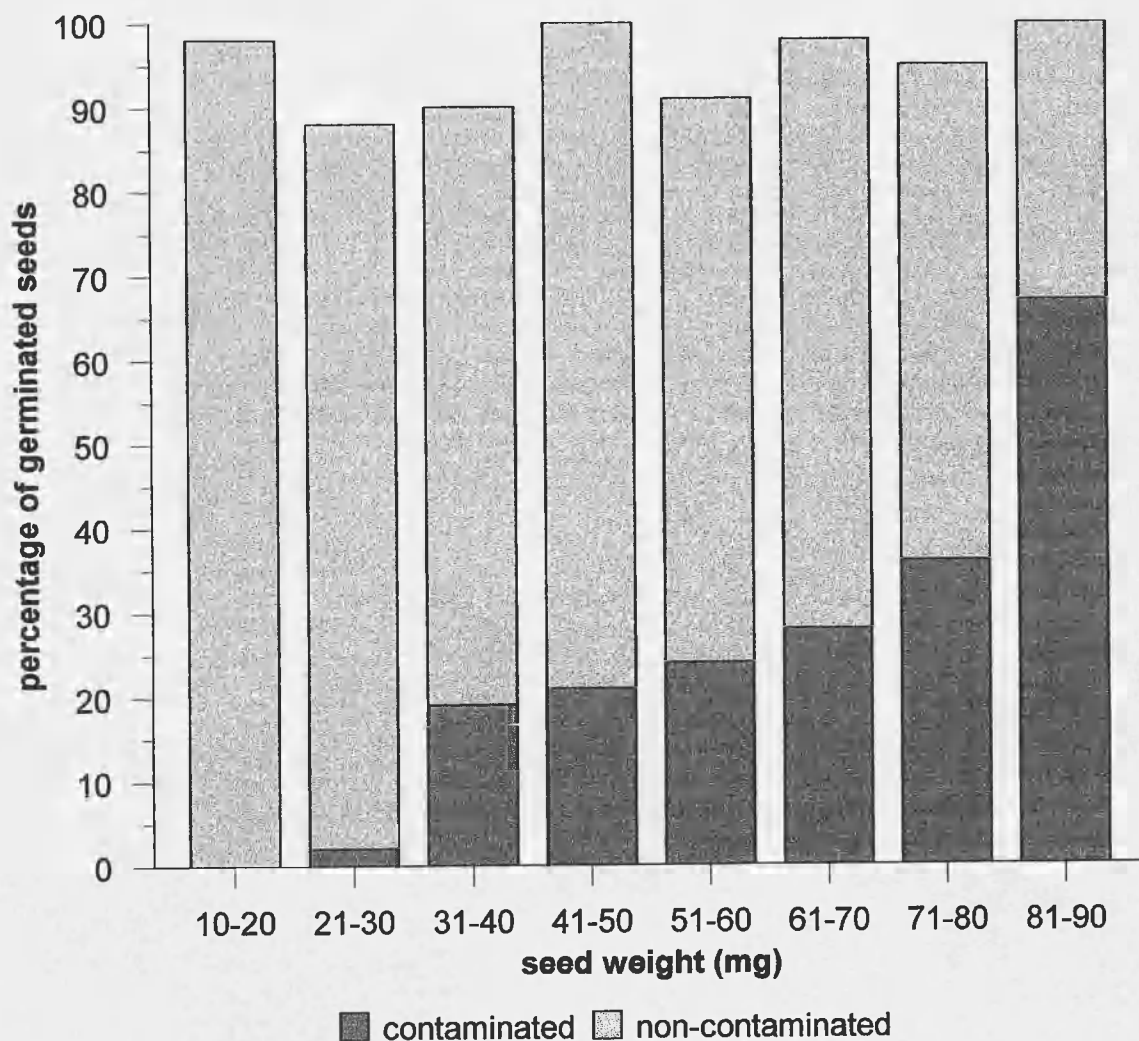


Figure 6 The percentage of *Microdochium nivale* contaminated and non-contaminated wheat seed (lot No. 15, see Table 3) in each weight category that had germinated on potato dextrose agar after 14 days at 15°C.

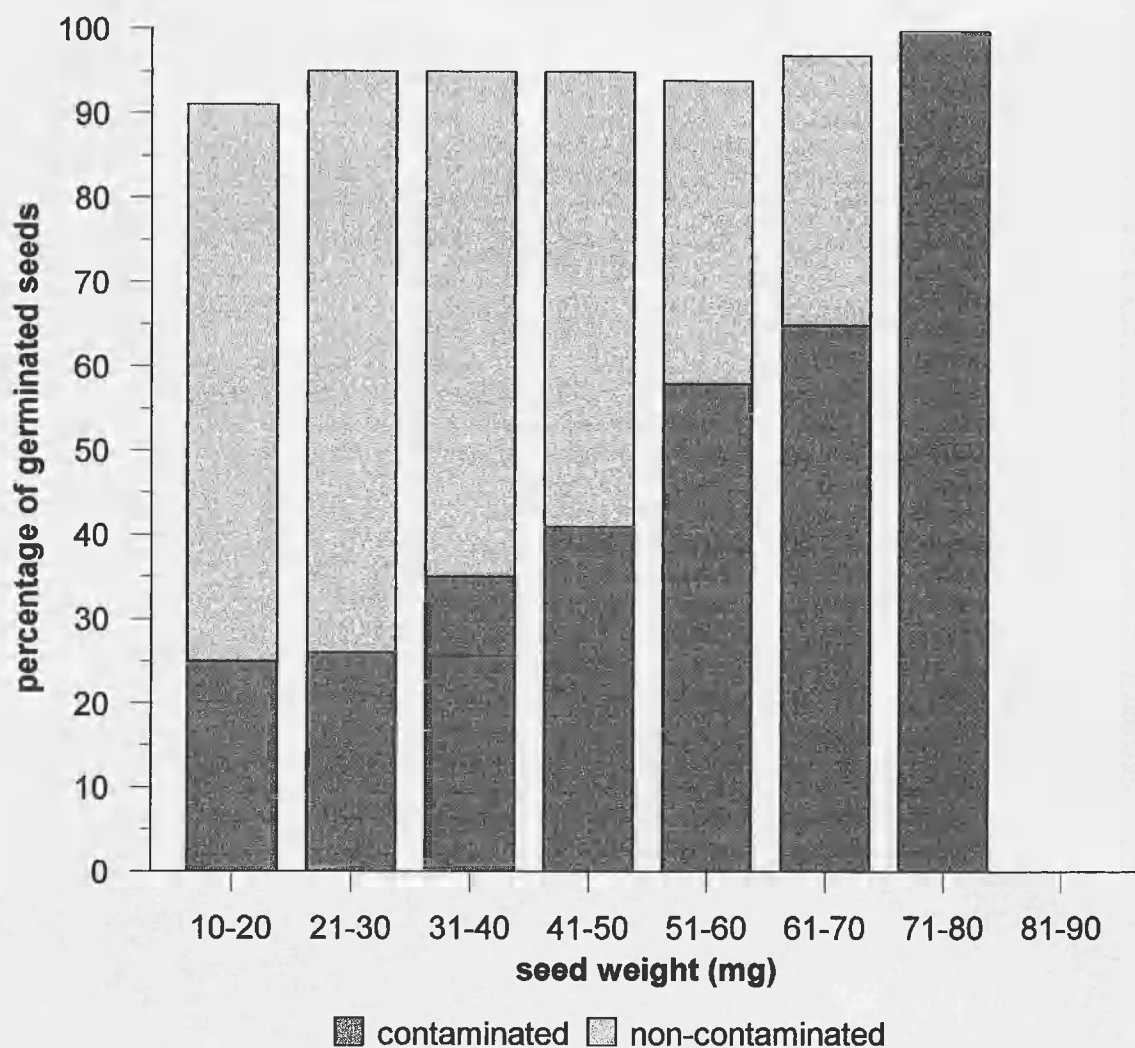


Figure 7 The percentage of *Microdochium nivale* contaminated and non-contaminated wheat seed (lot No. 19, see Table 3) in each weight category that had germinated on potato dextrose agar after 14 days at 15°C.

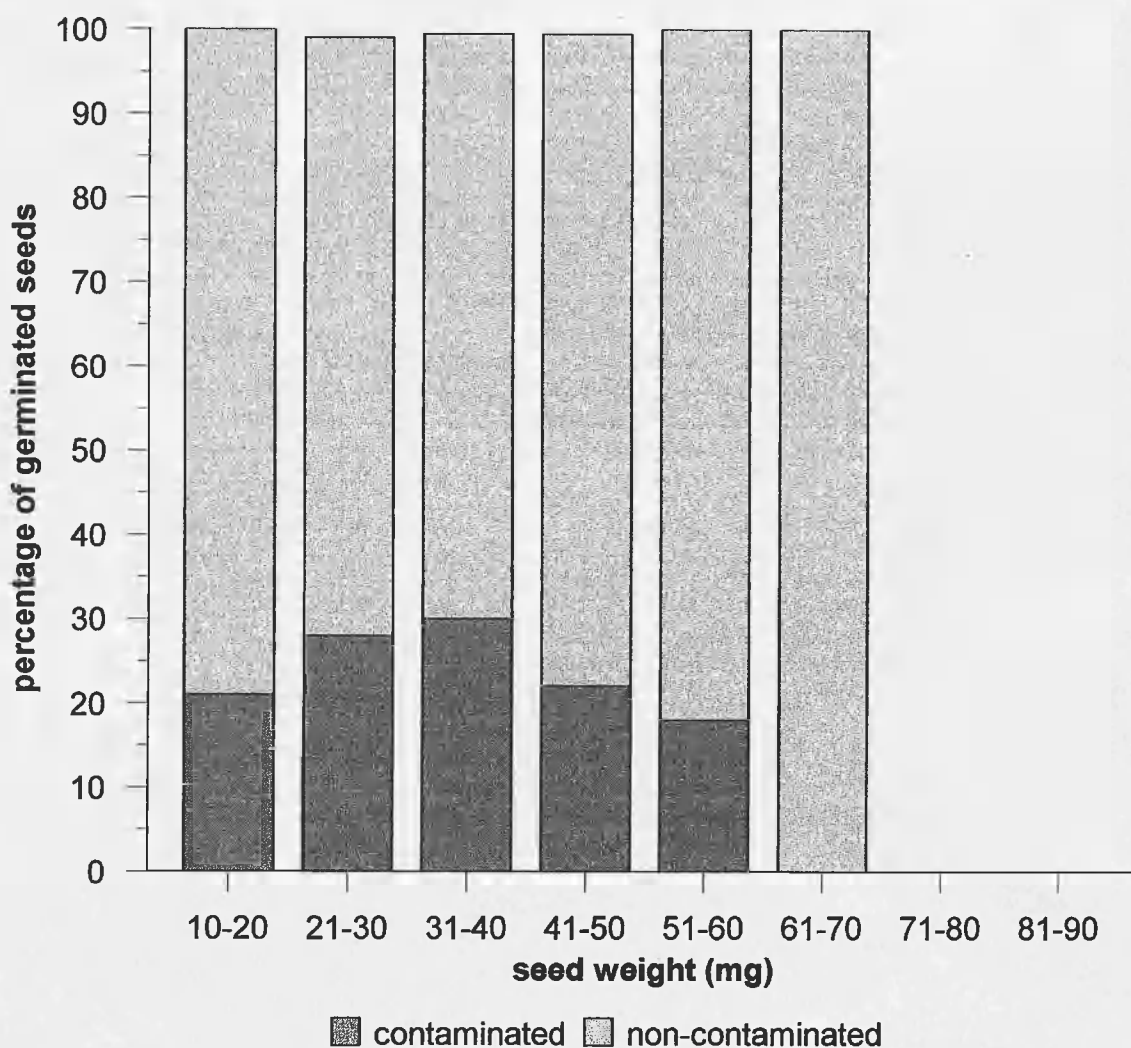


Figure 8 The percentage of *Microdochium nivale* contaminated and non-contaminated wheat seed (lot No. 18, see Table 3) in each weight category that had germinated on potato dextrose agar after 14 days at 15°C.

(ii) The Relationship between *M. nivale* Contamination, Seed Weight and the Emergence of Wheat Seedlings

Light seeds emerged significantly ($P < 0.001$) faster than heavy seeds (Table 12). On average light seeds emerged 1.4 days before heavy seeds.

In the absence of fungicide, significantly more ($P = 0.006$) light seeds emerged than heavy seeds. This effect was most pronounced between light and heavy seeds from seed lot number 19 where significantly ($P = 0.008$) more seedlings emerged from light seeds (82.5%) than from heavy seeds (57.5%) (Table 12).

The emergence of fungicide treated seed was good ($> 90\%$) for both light and heavy seed with the exception of heavy seed (74%) from seed lot number 19 (Table 12).

(iii) The Relationship between *M. nivale* Seed Contamination, Seed Weight and the Severity of *Fusarium* Seedling Blight Symptoms

Only data from treatments where no fungicide was used were analysed statistically, as guazatine treatment of seed resulted in almost total elimination of disease symptoms (maximum disease score recorded 0.036).

In the absence of fungicide, significantly higher disease scores were given to seedlings from heavy seed than to seedlings from light seed (Table 12). The mean seedling disease score from all contaminated lots was 1.21 for heavy seeds and 0.60

for light seeds.

(iv) The Relationship between *M. nivale* Seed Contamination, Seed Weight and Seedling Growth

Root length

Heavy seeds produced seedlings with longer roots than light seeds and this relationship was observed for seeds either treated with or without fungicide ($P < 0.001$) (Table 12). On average, treatment with fungicide did not result ($P = 0.785$) in an increase in root length and a relationship between seed weight, fungicide treatment and a reduction in root length caused by the pathogen is not easily seen.

Coleoptile length

Microdochium nivale seed contamination significantly reduced coleoptile length ($P < 0.001$). The mean length of coleoptiles, for both heavy and large seeds with or without seed treatment, decreased from 34.3 mm to 29.9 mm as the number of contaminated seeds increased. The effect of seed weight on coleoptile length was not significant ($P = 0.793$), and neither was there an interaction between seed weight and seed contamination ($P = 0.310$) (Table 12).

Shoot length

Microdochium nivale seed contamination, seed weight and fungicide treatment all had a significant effect on shoot length ($P < 0.001$). Guazatine treatment resulted

Table 12 The relationship between the weight of *Microdochium nivale* contaminated wheat seeds (Table 3) and guazatine seed treatment with respect to their effect on the emergence and subsequent growth of seedlings at 6°C.

seed lot No.	seed weight category*	rate of seedling emergence (1/days)	No. of emerged seedlings	root length (mm)	coleoptile length (mm)	first leaf length (mm)	seedling dry weight (g)	disease score
<u>untreated seed</u>								
15	heavy	0.0466	4.0	84.1	25.9	105.9	0.198	1.40
15	light	0.0537	5.0	73.7	27.3	111.4	0.077	0.96
19	heavy	0.0464	9.2	92.9	29.0	107.8	0.414	0.88
19	light	0.0487	13.2	76.2	30.4	108.9	0.240	0.31
18	heavy	0.0540	11.8	89.1	30.8	120.0	0.474	1.36
18	light	0.0520	13.4	70.0	29.2	103.4	0.171	0.53
22	heavy	0.0550	15.6	79.8	36.2	139.6	0.667	0.00
22	light	0.0574	15.6	61.4	33.0	124.7	0.256	0.13
<u>guazatine treated seed</u>								
15	heavy	0.0544	15.8	79.3	35.3	125.0	0.784	
15	light	0.0588	14.6	89.1	31.1	117.3	0.235	
19	heavy	0.0442	11.8	91.8	31.6	118.5	0.542	
19	light	0.0524	14.2	74.4	32.8	115.5	0.252	
18	heavy	0.0562	15.4	82.7	33.9	127.3	0.652	
18	light	0.0558	15.2	71.9	32.3	114.6	0.205	
22	heavy	0.0559	15.2	72.8	35.3	136.2	0.569	
22	light	0.0642	15.8	61.6	32.6	131.8	0.276	
SEM		0.00148	0.71	4.28	1.32	3.56	0.0250	0.246
D of F		64	64	64	64	64	64	32
CV		6.2	12.4	12.2	9.3	6.5	15.0	7.2

* for specific mean seed weights see Table 11.

in an increase in mean shoot length. The mean shoot length from treated seed was 115 mm and from untreated seed 123 mm. There was a significant interaction between seed contamination and seed weight ($P = 0.014$) making the overall effects of contamination and weight less clear. The shoot height of seedlings from seeds treated with guazatine decreased with increasing seed contamination, with heavy seeds producing seedlings with longer shoots than light seeds. In contrast, when seeds were not treated with guazatine, heavy seeds from lots with no *M. nivale* contamination (No. 22) or low contamination (No. 18) produced seedlings with longer shoots than light seeds. When the percentage of *M. nivale*-contaminated seeds was higher (seed lot No. 15 and 19), light seeds produced seedlings with longer shoots than heavy seeds (Table 12).

Seedling dry weight per pot

Microdochium nivale seed contamination, seed weight and fungicide treatment all had a significant effect on the mean dry weight of seedlings per pot ($P < 0.001$). Mean seedling dry weight was greater for seedlings produced from heavy seeds than from light seeds, and treatment with guazatine increased seedling dry weight when applied to contaminated seed (Table 12).

DISCUSSION

This work has shown that there is no clear relationship between natural contamination of wheat seed by *M. nivale* and the weight of individual wheat seeds. Within each seed lot, contaminated and non-contaminated seeds were distributed by weight in similar near normal distributions. It is often assumed, although there is no published evidence, that, like *Fusarium* spp., the *M. nivale*-contaminated component of a seed lot is comprised mainly of smaller, lighter seeds. However, in this study, small light seeds were as likely to be contaminated by *M. nivale* as large heavy seeds. Therefore, unlike *F. roseum*, where the “cleaning” of naturally contaminated seed lots was possible by the removal of small light seeds (Nakagawa and Yamaguchi, 1989), *M. nivale* contaminated seed lots cannot be “cleaned” on the basis of seed size and weight.

Wheat seed contamination with *M. nivale* is often associated with reduced germination (Rennie *et al.*, 1990) and reduced emergence (Cockerell, 1995). In the investigation in this chapter the relationships between *M. nivale* seed contamination, seed weight and wheat seed germination and emergence were different. More heavy contaminated seeds germinated than light seeds, although more seedlings emerged from light contaminated seeds than from heavy ones. The nutrient status of the growing medium was demonstrated by Shen (1940) to have an effect on the pre-emergence death of wheat seedlings. Fewer seedlings were killed when grown in the absence of nutrients. The effect of nutrient status of the growth medium may go some

way to explain the differences observed between the PDA and soil experiments. The germination test was performed on a nutrient rich medium (PDA), whilst seedling emergence was assessed in a soil-based compost presumed to be of lower nutrient status. The high nutrient medium in the germination experiment may have favoured the growth of the fungus, which relies on an external supply of nutrients. With a large supply of nutrients in the PDA, small light seeds could be overgrown rapidly by the fungus. However, in a soil-based compost with a much reduced nutrient status the fungus would be more reliant on the seed as a food source. Heavy seeds with large endosperms would provide more nutrients for fungal growth and subsequent seedling attack than small light seeds.

Seedlings from small light wheat seeds emerged more quickly than from larger heavy seeds, on average reaching mean emergence 1.4 days earlier. The faster emergence of seedlings from small wheat seeds has been recorded by Lafond and Baker (1986a). Lafond and Baker studied the effect of seed size on the speed of seedling emergence in the field in two consecutive years using nine spring wheat cultivars. They found that small, light seeds of mean weight 26.3 - 33.1 mg (year 1) and 25.9 - 45.3 mg (year 2) emerged faster than larger heavier seeds 35.8 - 45.7mg (year 1) and 39.3 - 49.8 mg (year 2) irrespective of the cultivar used. In addition, subsequent studies using the same wheat cultivars (Lafond and Baker, 1986b) showed that small light seeds germinated faster than larger heavier seeds. In the work reported here, it appeared that the faster seedlings emerge, the greater their chance of escaping infection. Microscopic investigation of the mycelial growth of *M. nivale* and the

subsequent infection of light and heavy seeds placed in a range of media may provide information as to the cause of these observations.

Disease symptoms on emerged seedlings were, on average, more severe on seedlings from heavy seeds than from light seeds despite seedlings from heavy seeds appearing to be more vigorous. Heavy seeds produced seedlings with a mean weight of 0.045 g and light seeds produced seedlings with a mean weight of 0.016 g.

The relationship between *M. nivale* contamination, seed weight and the growth of seedlings was complex. Overall, there was no clear relationship between *M. nivale* contamination, seed weight and seedling growth. Heavy seeds treated with guazatine produced virtually disease free seedlings with longer shoots than lighter, treated seed, for each of the seed lots used. A similar observation was made by Lafond and Baker (1986a). In field trials using nine different cultivars of spring wheat over two years they observed that seedlings from heavy seed accumulated more shoot dry weight than seedlings from light seed. However, in the present study, when seed was not treated with guazatine, heavy seeds produced seedlings with the longest shoots only when seed contamination was low. When the percentage of seeds contaminated by *M. nivale* was high, seedlings from light seeds had the longest shoots. It appears therefore, that seedlings from small light contaminated seeds were not as severely affected by *M. nivale* infection as seedlings from larger heavier contaminated seeds.

It is known that small, light wheat seeds produce smaller less vigorous seedlings than heavy wheat seeds (Lafond and Baker, 1986a). It has also been

reported that small wheat seeds are the major *Fusarium*-contaminated component of a batch (Bechtel *et al.*, 1985; Nakagawa and Yamaguchi, 1989). On this basis small seeds could be removed from a batch of *Fusarium* contaminated wheat leaving just the larger more vigorous seeds which are free from contamination. However, it can be seen from the work presented here that the removal of small, light seed from a batch of wheat naturally contaminated by *M. nivale* would not reduce the percentage of seeds contaminated by *M. nivale*. In addition, in the absence of a fungicide seed treatment, the selection of larger seed for sowing could result in lower seedling emergence and more severe post-emergence disease symptoms than if the "uncleaned" batch was sown.

CHAPTER 5

The effect of temperature and soil water on the severity of *Fusarium* seedling blight and the relationship between the rate of seedling emergence and pre- emergence seedling death.

INTRODUCTION

It is clear from previous studies that temperature and soil water have a marked effect on the severity of *Fusarium* seedling blight symptoms (Dickson, 1923; Shen, 1940; Colhoun and Park, 1964; Colhoun *et al.*, 1968; Millar and Colhoun, 1969 and Duben and Fehrmann, 1979), and that there is some evidence from work with *F. culmorum* and wheat seedlings to suggest that the rate of seedling emergence is closely related to the subsequent severity of seedling blight symptoms (Malalasekera and Colhoun, 1968). However, little work has been performed with wheat seed naturally contaminated by *M. nivale* and little is known about the relationship between the rate of emergence of seed naturally contaminated by *M. nivale* and pre-emergence seedling death.

The aim of this work was to investigate the effect of temperature and soil water on the pre-emergence death of seedlings and to investigate the relationship between the rate of seedling emergence and pre-emergence seedling death for seed artificially inoculated with either *M. nivale* or *F. culmorum* macroconidia and seed naturally contaminated by *M. nivale*.

In the previous studies (Dickson, 1923; Shen, 1940; Colhoun and Park, 1964; Colhoun *et al.*, 1968; Millar and Colhoun, 1969; Cassell and Hering, 1982) the maximum value of soil water content either maximum water holding capacity (MWHC) or soil moisture content (θ_m) measured gravimetrically and expressed as a percentage by mass, was used to represent the soil water status over the entire period

of each experiment. Colhoun and Park (1964) and Colhoun *et al.* (1968) assessed the effects of soil water using three soils with decreasing MWHC. Pots containing soil were weighed at the start of their experiments and rewatered to their original weight three times a week. In contrast, Cassell and Hering (1982) used soils that were watered daily to a maximum soil water content (w/w). However, Oyarzun *et al.* (1994) observed that watering the surface of soil to a maximum soil water value can result in large differences between maximum and minimum soil water values. In addition, this difference in soil water would increase as the interval between soil watering was lengthened. Therefore, if a maximum value of soil water is used and water is lost from the soil by plant uptake or evaporation from the soil surface, the maximum value will not be maintained without continuous correction for the losses. If continuous correction is not performed then the use of the maximum value of soil water, as a measure of the soil water status over the entire experiment seems unjustifiable.

When investigating the effect of soil water on the infection of wheat coleoptiles by *F. graminearum*, Liddell and Burgess (1988) minimised water loss from the soil by plant uptake and evaporation by using thin wax layers placed in the soil above germinating seeds and on the surface of the soil. This technique allowed the maintenance of a constant soil water value in the enclosed soil. However, the technique was only considered suitable for the study of coleoptile infection in soils containing the pathogen because of limitations resulting from the long time required for soil water equilibration, the possible reduction in oxygen supply caused by the

wax layers, and the requirement for the exclusion of plant roots from the enclosed soil. This method is therefore of little use when investigating the effect of soil water on seed-borne pathogens and the subsequent emergence of wheat seedlings.

In the course of the present investigations it was realised that the method used to maintain soil water (Colhoun and Park, 1964) was not very precise or easily reproducible. An investigation to ascertain the most appropriate measure of soil water and how best to maintain this value was investigated in simple pot experiments. The results of this investigation will be presented first in this chapter.

The value used to measure soil water is also important. Previous workers used MWHC and θ_m as measurements of soil water status. However, these are unique to the individual soils, and it is therefore impossible to repeat work accurately without using identical soil and watering regimes. Soil matric potential (ψ_m) was therefore selected as a measure of soil water for the present study as this measures the availability of water to plants and pathogens and can be readily compared between different soils (Griffin, 1969).

MATERIALS AND METHODS

(i) The Maintenance and Measurement of Soil Water and its Effect on *Fusarium* Seedling Blight of Wheat

Preparation of *F. culmorum* inoculum

A conidial suspension of *F. culmorum* was prepared by washing macroconidia from a sporulating culture of *F. culmorum* grown on PDA with 5 ml of sterile distilled water. The resultant conidial suspension was then passed through three layers of muslin to remove hyphal fragments and centrifuged at 5000 r.p.m. for 10 minutes. Following centrifugation the supernatant was decanted and the conidia resuspended in sterile distilled water. The concentration of conidia was determined using a haemocytometer and adjusted to give a suspension of 10^4 conidia ml⁻¹ of water.

Inoculation and sowing of seed

Winter wheat seed cv. Mercia (lot No. 14, Table 3) was used in this study. Isolations from wheat seeds were taken to determine whether natural seed-borne *F. culmorum* contamination was present. One hundred seeds were placed, five to a Petri dish, onto PDA and incubated at 20°C. After 14 days the fungal colonies present were identified from their colony and spore morphology.

The germination potential of the seed was determined by placing seed onto

moist filter paper in Petri dishes. The seeds were incubated at 20°C for seven days and the number of germinated seeds recorded. Five Petri dishes each containing 50 seeds were used.

Before inoculation with conidial suspension, seeds were placed in sodium hypochlorite solution (1% available chlorine) for three minutes, washed three times in sterile distilled water and then dried in a flow of sterile air. These surface sterilized seeds were then inoculated with macroconidia at the rate of 10^4 spores (25 g)⁻¹ of seed. The seed and conidial suspension were mixed together in a 250 ml conical flask and shaken for three minutes. Non-inoculated surface sterilized seeds were similarly treated, but with sterile distilled water to provide controls.

Seeds were planted evenly at a depth of 2 cm into a plastic tray (165 x 95 x 45 mm) containing 480g of steam-sterilized sandy loam based compost (John Innes No.2) at a rate of 20 seeds per tray (Table 4). Three replicate trays were used for each inoculated and non-inoculated treatment.

Maintenance of soil water

In order to provide soil water conditions conducive to seedling emergence (Khah *et al.*, 1986), mean θ_m was restricted to between 10% and 18% (w/w). As Khah *et al.* had worked in a sandy loam soil, John Innes (No. 2) compost was selected for this study because of its sandy texture and to allow repeatability and continuity with future experiments.

In order to determine the initial θ_m of the soil before watering, compost was

first weighed and then oven dried at 102°C to a constant weight (Anon., 1977a). The quantity of water added to each tray was regulated gravimetrically. Sterile distilled water was added to the surface of the compost using a fine nozzle wash bottle until the required weight of water had been added. The mean soil water status of the soil for each watering treatment was calculated from the maximum and minimum values recorded at watering. Table 13 shows the weight to which the trays were watered and the times of watering. Treatments 1 to 6 were watered to similar maximum soil water values, whilst treatments 7 to 12 were watered to obtain similar mean soil water values equivalent to the maximum values used in treatments 1-6. A further twelve treatments (13-24) were used to provide controls. These treatments were identical to treatments 1-12 except for the use of non-inoculated seeds. Each watering treatment was replicated three times.

A soil water retention curve, relating θ_m to ψ_m for the compost was obtained by the pressure plate method (Anon., 1977b) (see Chapter 2). Table 14 shows the maximum and average ψ_m produced by each watering treatment. Large differences in soil water content were produced by the watering regimes used, although these related to a more restricted range of ψ_m values owing to the shape of the soil water retention curve. A similar soil water retention curve was produced by Wadleigh *et al.* (1946) for Panoche sandy loam soil. Measurements of ψ_m were made on the drying curve. In theory, owing to hysteresis, these measurements do not represent fully the relationship between θ_m and ψ_m in a drying wetting cycle, although the sandy nature of the compost and its constant bulk density throughout the experiment would limit

this effect. In addition, Liddell and Burgess (1988) showed that the influence of hysteresis on the soil moisture content and soil moisture potential relationship was not important in the study of infection of wheat coleoptiles by *F. graminearum* Group 1.

Environmental conditions

Trays were placed, according to a fully randomised design, in two growth cabinets (Convion 510H) at a constant temperature of 20°C and at 75% relative humidity. The trays were re-randomised daily. A photoperiod of ten hours was provided by 80 W cool white fluorescent lamps supplemented by 40 W tungsten lamps giving a mean photon flux density of 180 $\mu\text{Em}^{-2}\text{s}^{-1}$.

Assessment of the rate of seedling emergence

The number of emerged seedlings per tray was counted daily until no further seedlings emerged on five successive days. No subsequent emergence was seen. The mean seedling emergence time in days was calculated using Equation 1 as previously described (Khah *et al.*, 1986) (Chapter 2).

Table 13 The maximum weight to which trays were watered and the timing of watering. The controls (treatments 13-24) were watered in the same way as their inoculated treatments.

Treatment number	Time of watering ^a			Weight to which trays were watered (g)
	0900 h	1400 h	1900 h	
1	✓	✓	✓	513
2	✓	X	✓	513
3	X	X	✓	513
4	✓	X	X	513
5	✓	X	X	513
6	✓	X	X	513
7	✓	✓	✓	516
8	✓	X	✓	518
9	X	X	✓	520
10	✓	X	X	522
11	✓	X	X	528
12	✓	X	X	540

^a waterings were performed daily except for treatments 5 and 11 which were watered every second day and treatments 6 and 12 which were watered every third day.

Table 14 Mean and maximum soil matric potentials (ψ_m) produced in John Innes compost number 2 at 20°C using different watering regimes. The controls (treatments 13-24) were watered in the same way as their inoculated treatments.

Treatment number	Soil matric potential (MPa)	
	Max.	Mean
1	-0.135	-0.138
2	-0.135	-0.140
3	-0.135	-0.148
4	-0.135	-0.150
5	-0.135	-0.163
6	-0.135	-0.175
7	-0.129	-0.135
8	-0.125	-0.135
9	-0.124	-0.135
10	-0.122	-0.135
11	-0.116	-0.135
12	-0.105	-0.135

Disease assessment

Seedling death following emergence was also recorded daily and the incidence of lesions on surviving plants was assessed 21 days after planting. The total

percentage incidence of infection was calculated by expressing the sum of the seedlings killed before and after emergence and the emerged seedlings with disease symptoms as a percentage of the number of seeds planted. *Fusarium culmorum* infection of diseased seedlings was confirmed by isolation on PDA following surface sterilization for three minutes in sodium hypochlorite (1% available chlorine) and three rinses in sterile distilled water.

Data analysis

Data were statistically examined using linear regression analysis to investigate the effect of soil matric potential on *Fusarium* seedling blight disease symptoms and to investigate the relationship between the rate of seedling emergence and pre-emergence seedling death. Statistical analysis was performed on a personal computer using the software program Statgraphics.

(ii) The Effect of Temperature and Soil Water on the Pre-emergence Death of Seedlings Following Inoculation with *M. nivale* and the Relationship between the Rate of Seedling Emergence and Pre-emergence Seedling Death

Preparation of *M. nivale* inoculum

A conidial suspension of *M. nivale* was prepared by washing macroconidia from a sporulating culture of *M. nivale* grown on potato dextrose agar (PDA) with

5 ml of sterile distilled water. The resultant conidial suspension was then passed through three layers of muslin to remove hyphal fragments and centrifuged at 5000 r.p.m. for 10 minutes. Following centrifugation the supernatant was decanted and the conidia resuspended in sterile distilled water. The concentration of conidia was determined using a haemocytometer and adjusted to give a suspension of 10^5 conidia ml^{-1} of water.

Inoculation and sowing of seed

Winter wheat seed cv. Mercia (lot No. 14, Table 3) was used in this study. Isolations from wheat seeds were taken to determine whether natural seed-borne *M. nivale* contamination was present (Chapter 2).

The germination potential of the seed was determined by placing seed onto moist filter paper in Petri dishes. The seeds were incubated at 20°C for seven days and the number of germinated seeds recorded. Five Petri dishes each containing 50 seeds were used.

Before inoculation with conidial suspension, seeds were placed in sodium hypochlorite solution (1% available chlorine) for three minutes, washed three times in sterile distilled water and then dried in a flow of sterile air. These surface sterilized seeds were then inoculated with macroconidia at the rate of 10^5 spores 25 g^{-1} of seed. The seed and conidial suspension were mixed together in a 250 ml conical flask and shaken for three minutes. Non-inoculated surface sterilized seeds were similarly treated, but with sterile distilled water to provide controls.

Seeds were planted evenly at a depth of 2 cm into a plastic tray (215 x 155 x 45 mm) containing 950 g of steam-sterilized sandy loam based compost (John Innes No.2) at a rate of 35 seeds per tray (Table 4). Four replicate trays were used for each inoculated and non-inoculated treatment.

Soil water treatments

In order to determine the initial θ_m of the soil before watering, compost was first weighed and then oven dried at 102°C to a constant weight (Anon., 1977a). The quantity of water added to each tray was regulated gravimetrically. Sterile distilled water was added to the surface of the compost daily using a fine nozzle wash bottle until the required weight of water had been added. The mean soil water status of the soil for each watering treatment was calculated from the maximum and minimum values recorded at watering. Trays were watered to maximum θ_m values of 16%, 22% and 24% to give low, medium and high soil water treatments in each of the three temperatures. A soil water retention curve relating θ_m to ψ_m for the compost was obtained by the pressure plate method (Anon., 1977b) (see chapter 2). Table 15 shows the mean ψ_m produced by each low, medium and high soil water treatment at each temperature.

Environmental conditions

Trays were placed, according to a fully randomised design into one of three growth cabinets (Convion 510H) according to temperature treatment. The mean

temperatures for each of the three growth cabinets used were 8.6°C, 12.2°C and 17.2°C. The mean temperatures were calculated from the duration of the “day time” and “night time” temperatures used, which were 10.0°C and 7.5°C, 15.0°C and 10.0°C and 20.0°C and 15.0°C respectively. A photoperiod of ten and a half hours was provided by 80 W cool white fluorescent lamps supplemented by 40 W tungsten lamps giving a mean photon flux density of 180 $\mu\text{Em}^{-2}\text{s}^{-1}$. Relative humidity within the cabinets was maintained at 75% during the duration of the experiment.

Table 15 The mean soil moisture (ψ_m)produced by each low, medium and high soil water treatment at each temperature.

Mean temperature (°C)	Mean soil ψ_m (MPa)		
17.2	-0.37	-0.13	-0.12
12.2	-0.19	-0.12	-0.11
8.6	-0.15	-0.11	-0.09

Assessment of seedling emergence

The number of emerged seedlings per tray was counted daily until no further seedlings emerged on five successive days. No subsequent emergence was seen. The mean seedling emergence time in days was calculated using Equation 1 as previously described (Khah *et al.*, 1986) (Chapter 2). The mean rate of emergence was calculated

as the reciprocal of the mean seedling emergence time in days .

Data analysis

Data were statistically examined using linear and multiple linear regression analysis. The effects of temperature and soil matric potential on seedling emergence and the rate of seedling emergence were described using multiple linear regression analysis. The relationship between the rate of seedling emergence and final seedling emergence was investigated using linear regression analysis. Statistical analysis was performed on a personal computer using the software program Statgraphics.

(iii) The Effect of Temperature and Soil Water on the Pre-emergence Death of Seedlings Caused by Natural *M. nivale* Contamination of Winter Wheat Seed and the Relationship between the Rate of Seedling Emergence and Pre-emergence Seedling Death

Natural *M. nivale* seed contamination and the sowing of seed

Winter wheat seed cv. Riband contaminated by *M. nivale* (lot No. 15, Table 3) and without *M. nivale* contamination (lot No. 22, Table 3) were used in this study. Isolations from a sample of each of the two seed lots were taken to determine the percentage of seeds naturally contaminated by *M. nivale*. This determination was on the basis of the growth of the pathogen from seeds placed on PDA (see Chapter 2).

The germination potential of the seed was determined by placing seed treated with the fungicide guazatine (600 μg of ai g^{-1} of seed) (Rennie and Gorey, 1988 and Cockerell, 1995) onto moist filter paper in Petri dishes. The seeds were incubated at 20°C for seven days and the number of germinated seeds recorded. Owing to the adverse effect of *M. nivale* on the normal development of seedlings, seed germination was assumed if any evidence of viability was seen prior to disease symptoms developing. Five Petri dishes each containing 50 seeds were used.

Seeds were planted evenly at a depth of 2 cm into a wide neck glass jar (60x60x120 mm) containing 60 g of steam sterilized sandy loam based compost (John Innes seed) adjusted to the required soil water content, at a rate of ten seeds per jar (Table 4). Ten replicate bottles were used for each treatment for contaminated and non-contaminated seed.

Temperature and soil water treatments

Four temperature (6°C, 8°C, 10°C and 12°C) and three soil water (-0.005 MPa, -0.036 MPa and -0.108 MPa) treatments were used in this study. Constant temperatures were maintained using four incubators set at 6, 8, 10 and 12°C respectively.

Following steam sterilization (see Chapter 2) the compost was allowed to dry in clean air in a laminar flow cabinet for 24 hours. The water content of the soil by mass (θ_m) was then determined by oven drying (Anon., 1977a) (see Chapter 2). Sterile distilled water was then added to the soil to raise the moisture content to either 21%,

23% or 27% by mass. The mean soil water content was calculated from the θ_m at the beginning and the end of the experiment and the mean soil matric potential (ψ_m) was obtained from the soil water retention curve for the compost, produced using the pressure membrane method (Anon., 1977b) (see Chapter 2).

Assessment of seedling emergence

The number of emerged seedlings in each jar was counted daily until no further seedlings emerged on five successive days. After this time no subsequent emergence was seen in any jar. The mean seedling emergence time in days was calculated using Equation 1 as previously described (Khah *et al.*, 1986) (Chapter 2).

Data analysis

The effect of temperature and soil water on seedling emergence and the rate of seedling emergence was investigated using ANOVA and multiple linear regression analysis. The relationship between the rate of seedling emergence and pre-emergence seedling death was examined using linear regression analysis. Statistical analysis was performed on a personal computer using the software program Statgraphics.

RESULTS

(i) The Maintenance and Measurement of Soil Water and its Effect on *Fusarium* Seedling Blight of Wheat

Seed-borne infection by *F. culmorum* or any other seed-borne pathogens was not detected in plated seed tests. The mean germination of seed on moist filter paper was 98% (± 0.63 ; $n = 5$). No seedling blight disease symptoms were seen in the non-inoculated control treatments. The mean emergence of non-inoculated seeds was 81% (± 5.69 ; $n = 36$).

The final emergence of inoculated seedlings was not found to be directly related to maximum ψ_m . In watering treatments 7 to 12 (Table 13), where the compost was watered to different maximum values whilst maintaining a similar mean ψ_m , no relationship ($R^2 = 0.01$; $P < 0.01$) was observed between emergence and maximum ψ_m . Mean emergence as a percentage of the controls for treatments 7 to 12 was 88% (± 2.18 ; $n = 6$) and the mean percentage of plants showing symptoms after emergence was 43% (± 1.20 ; $n = 18$). However, in treatments 1 to 6, where the compost was watered to the same maximum, but at different time intervals thereby producing different mean water contents, a strong relationship ($R^2 = 0.92$; $P < 0.01$) was observed between emergence and mean ψ_m (Figure 9). Figure 9 shows that as the mean ψ_m decreased from -0.138 to -0.175 MPa (ie. when the soil became drier), emergence of inoculated seedlings, as a percentage of the controls, decreased from

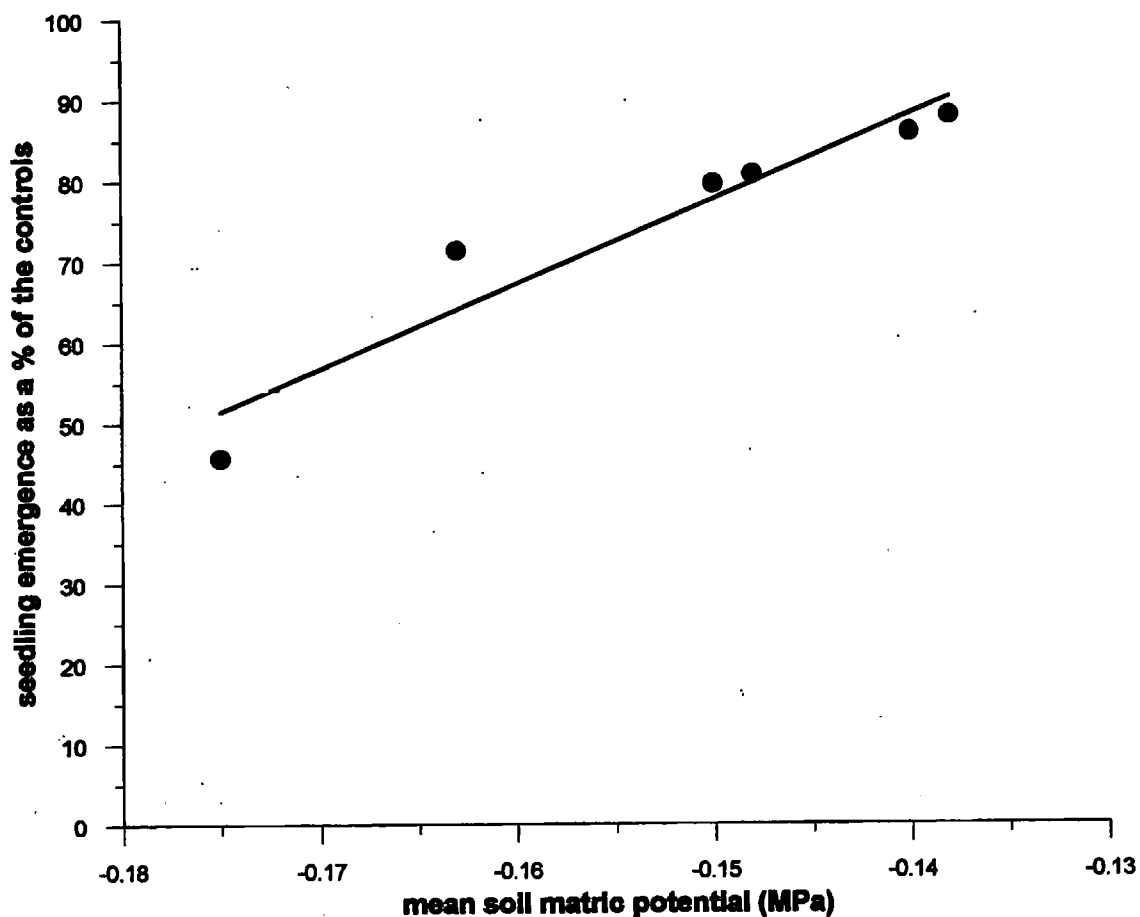


Figure 9 The effect of decreasing mean soil matric potential (Ψ_m) on the final emergence of wheat seedlings cv. Mercia (lot No. 14, see Table 3) inoculated with *Fusarium culmorum* macroconidia expressed as a % of the controls ($y = 1051x + 235$; $R^2 = 0.92$; $P < 0.01$). Values are means of three replicates.

88% to 47%. Seedlings emerged more slowly as mean ψ_m decreased. Figure 10 shows the relationship ($R^2 = 0.89$; $P < 0.01$) between mean ψ_m and the mean rate of seedling emergence of the non-inoculated controls. Seedlings emerged more quickly as the availability of water increased.

Figure 11 shows that the type of seedling blight symptoms observed, in the treatments where the soil was watered to a maximum ψ_m , was strongly influenced by mean ψ_m . Whilst pre-emergence death from seedling blight decreased linearly with increasing mean ψ_m ($R^2 = 0.92$; $P < 0.01$), the incidence of post-emergence symptoms, such as coleoptile lesions and extensive necrosis leading to seedling death, increased with increasing mean ψ_m ($R^2 = 0.85$; $P < 0.01$). Although the type of symptom observed was strongly influenced by the mean soil water potential, the total percentage incidence of infection, as indicated by the total percentage incidence of symptoms of any type, was relatively constant ranging from 73% to 85% and was not related ($R^2 = 0.01$; $P < 0.01$) to mean ψ_m .

(ii) The Effect of Temperature and Soil Water on the Pre-emergence Death of Seedlings Following Inoculation with *M. nivale* and the Relationship between the Rate of Seedling Emergence and Pre-emergence Seedling Death

Seed-borne infection by *M. nivale* or any other seed-borne pathogens was not detected in plated seed tests. The mean germination of seed on moist filter paper was

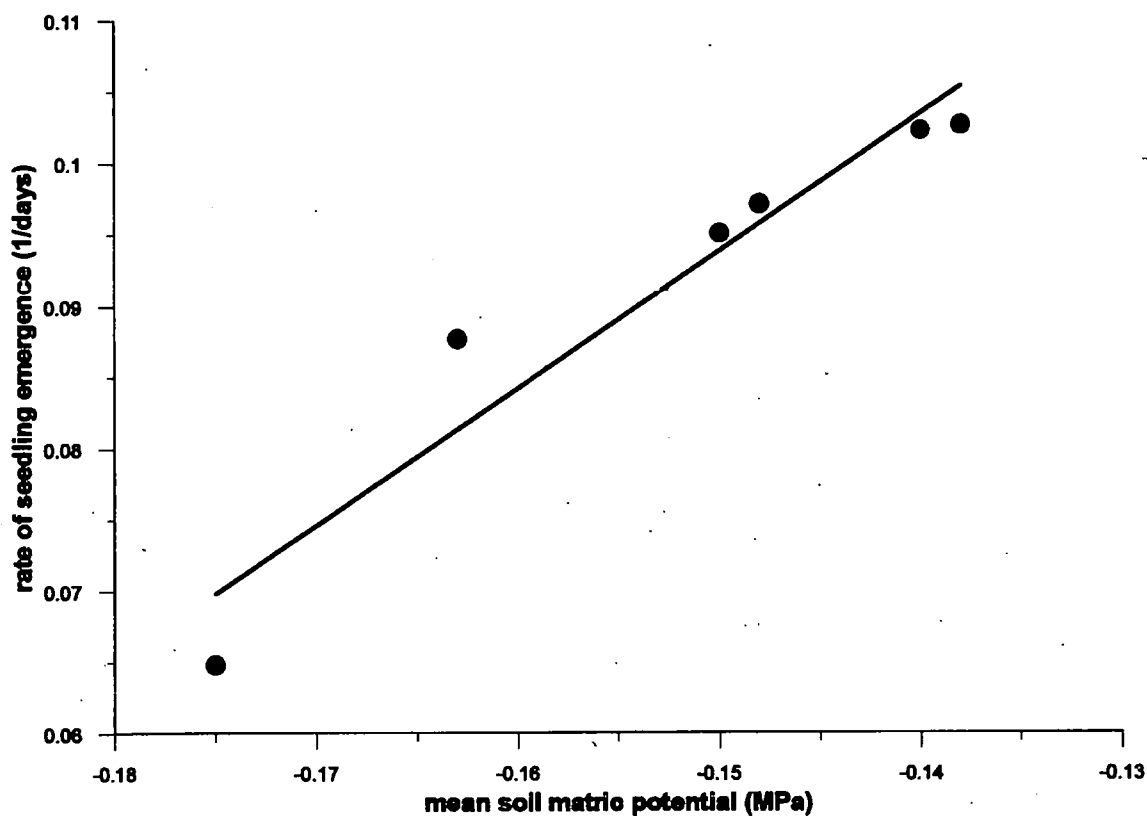


Figure 10 The relationship between mean soil matric potential (Ψ_m) and the mean rate of seedling emergence for non-inoculated wheat seedlings cv. Mercia (lot No. 14, see Table 3) ($y = 0.0962x + 0.238$; $R^2 = 0.89$; $P < 0.01$). Values are means of three replicates. SE are smaller than symbols.

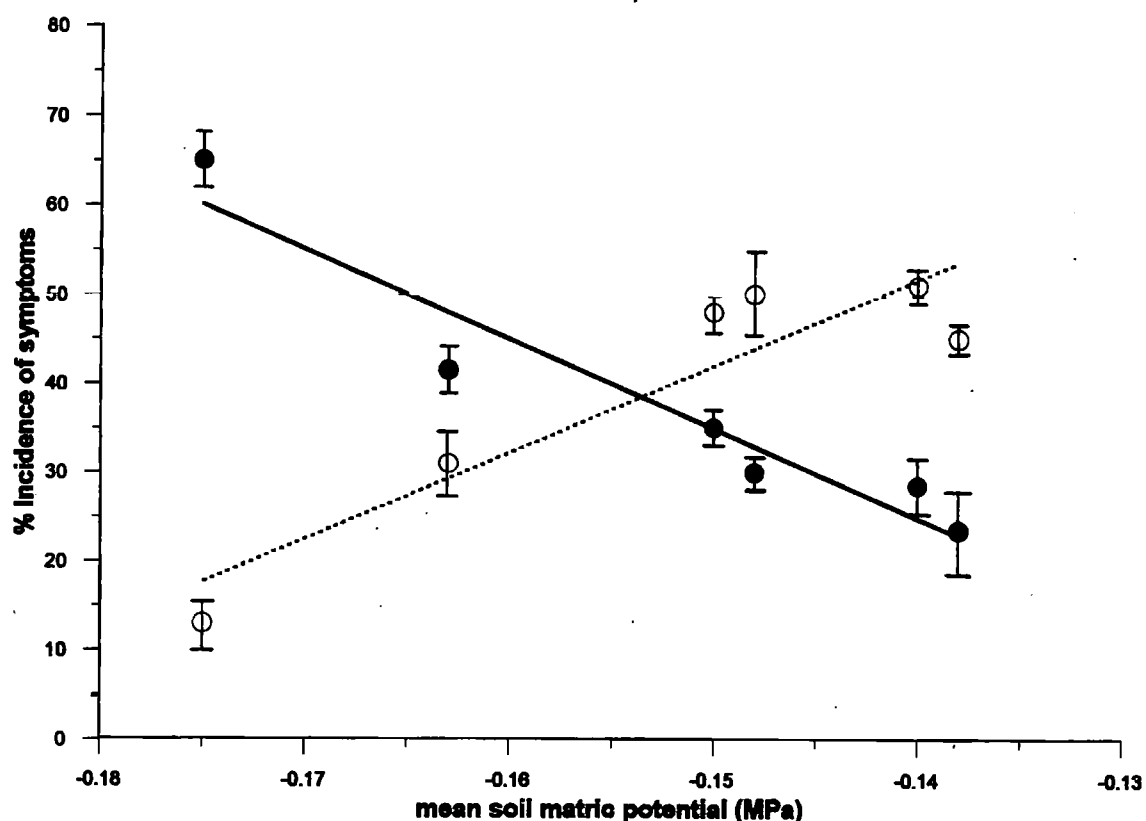


Figure 11 The effect of mean soil matric potential (Ψ_m) on pre-emergence death (●) ($y = -1007x - 116$; $R^2 = 0.92$; $P < 0.01$) and post-emergence symptoms (○) ($y = 968x + 187$; $R^2 = 0.85$; $P < 0.01$) caused by seed-borne *Fusarium culmorum* infection of winter wheat seedlings cv. Mercia (lot No. 14, see Table 3). Values are means of three replicates; bars indicate SE.

95% (± 0.65 ; $n = 5$).

Overall the emergence of inoculated seedlings as a percentage of the controls decreased with decreasing temperature and soil water (Figure 12). The greatest number of seedlings emerged at 17.2°C and -0.12 MPa and the fewest at 8.6°C and -0.15 MPa. At 17.2°C seedling emergence as a percentage of the controls declined from 79% to 64% as the soil matric potential was decreased from -0.12 MPa to -0.37 MPa, a difference of 0.25 MPa. At the lower temperatures, of 12.2°C and 8.6°C, seedling emergence also decreased with decreasing soil matric potential, but a greater decrease in emergence was seen for a smaller reduction in soil matric potential. Seedling emergence was reduced by 21% from 65% to 44% as the matric potential was reduced from -0.11 MPa to -0.19 MPa and by 20% from 32% to 12% as the matric potential was reduced from -0.09 MPa to -0.15 MPa at 12.2°C and 8.6°C respectively. Multiple linear regression analysis showed that there was a significant relationship between temperature, soil water and seedling emergence ($P < 0.001$) and that just over half of the variation seen in emergence at the different temperatures and soil water potentials was accounted for by the linear model ($R^2 = 0.58$).

The time taken for seedlings to emerge was also strongly affected by temperature and soil water, with seedlings emerging more quickly in warmer wetter soil. Overall the rate of seedling emergence increased from 0.050 days⁻¹ at 8.6°C and -0.15 MPa to 0.112 days⁻¹ at 17.2°C and -0.12 MPa (Figure 13). Multiple linear regression analysis showed that there was a significant relationship between temperature, soil water and the rate of seedling emergence ($P < 0.001$) and that three

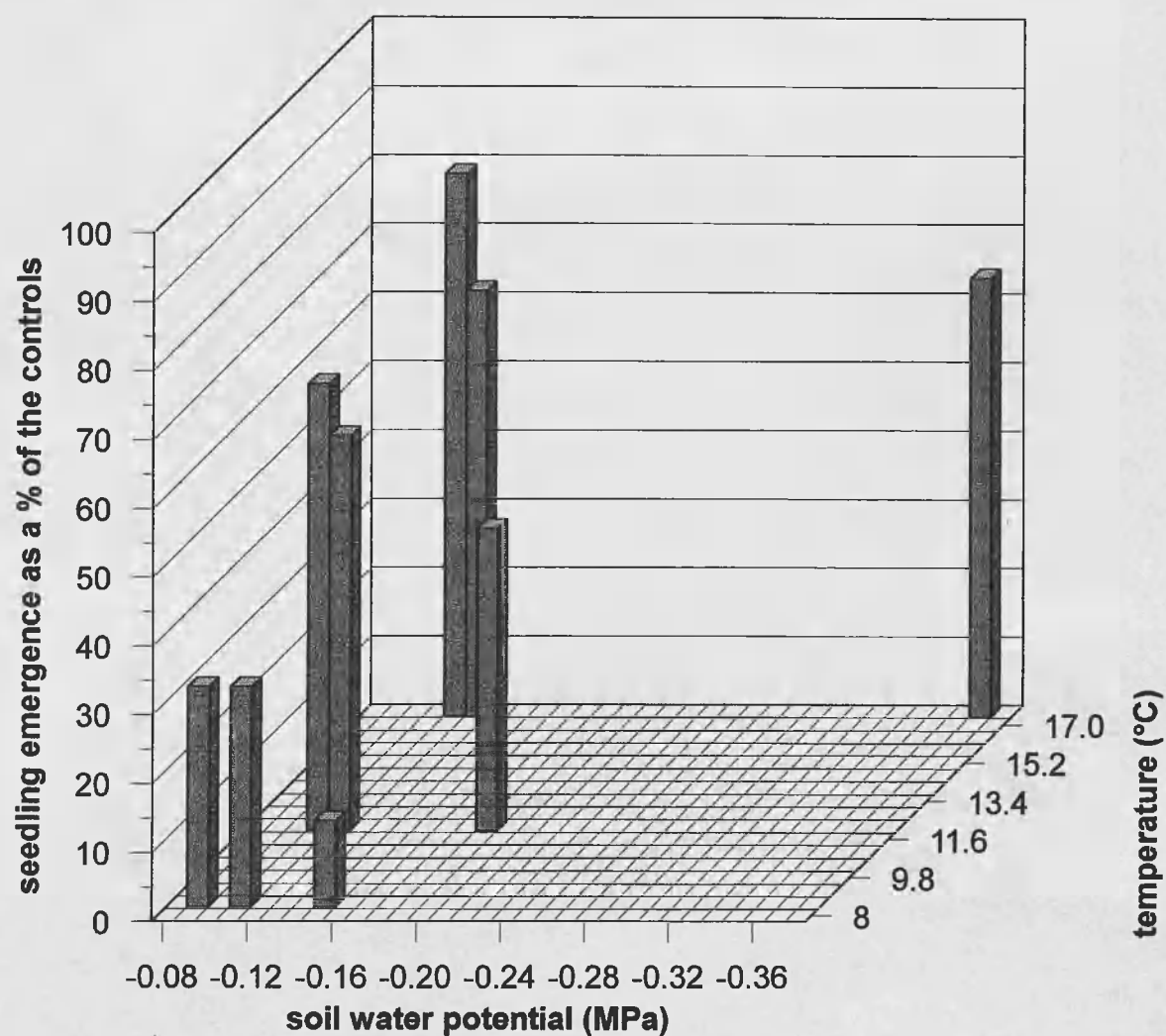


Figure 12 The effect of temperature and soil water potential on the emergence of wheat seedlings (lot No. 14, see Table 3) inoculated with *Microdochium nivale* conidia.

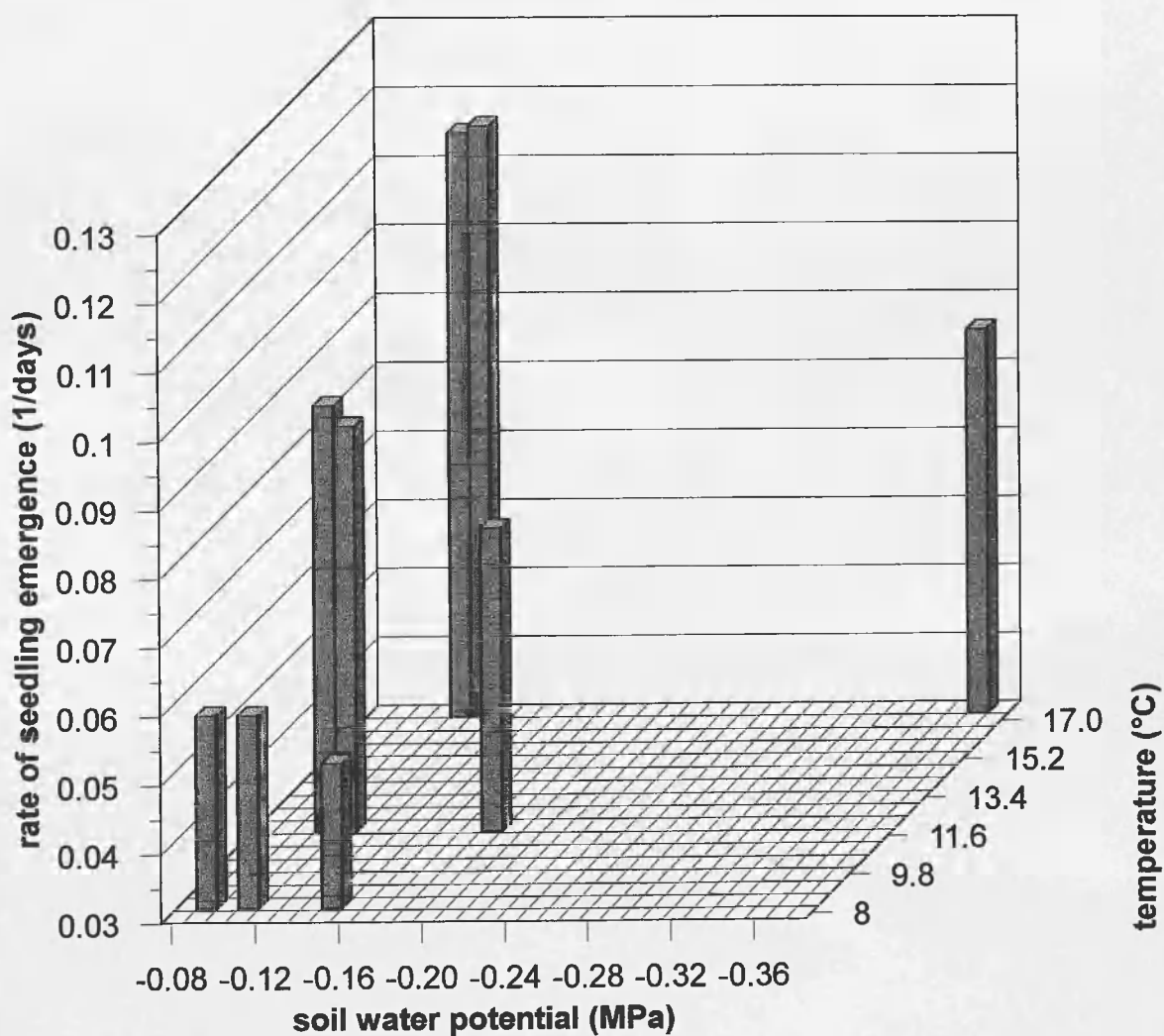


Figure 13 The effect of temperature and soil water potential on the rate of emergence of wheat seedlings (lot No. 14, see Table 3) inoculated with *Microdochium nivale* conidia.

quarters of the variation seen in the rate of seedling emergence at the different temperatures and soil water potentials was accounted for by the linear model ($R^2 = 0.76$).

The relationship between the time taken for inoculated seedlings to emerge and final seedling emergence was very strong. The number of seedlings that emerged decreased significantly ($P < 0.001$) in a linear fashion from 79% to 12 % as the time taken for seedlings to emerge increased from 8.9 days to 20 days (Figure 14).

(iii) The Effect of Temperature and Soil Water on the Pre-emergence Death of Seedlings Caused by Natural *M. nivale* Contamination of Winter Wheat Seed and the Relationship between the Rate of Seedling Emergence and Pre-emergence Seedling Death

Isolations on PDA revealed that 72% of seeds in seed lot number 15 (Table 3) were contaminated by *M. nivale* and that seed lot number 22 (Table 3) was free from *M. nivale* contamination. Seeds in both lots germinated well on moist filter paper. Ninety nine percent (± 0.01 ; $n = 5$) of seeds from lot number 22 and 94% (± 0.75 ; $n = 5$) of seeds from lot number 15 germinated.

Temperature and soil water had no effect on the final emergence of seedlings from seed that was not contaminated by *M. nivale* (lot No. 22). The final number of seedlings that emerged was constant between temperature and soil water treatments

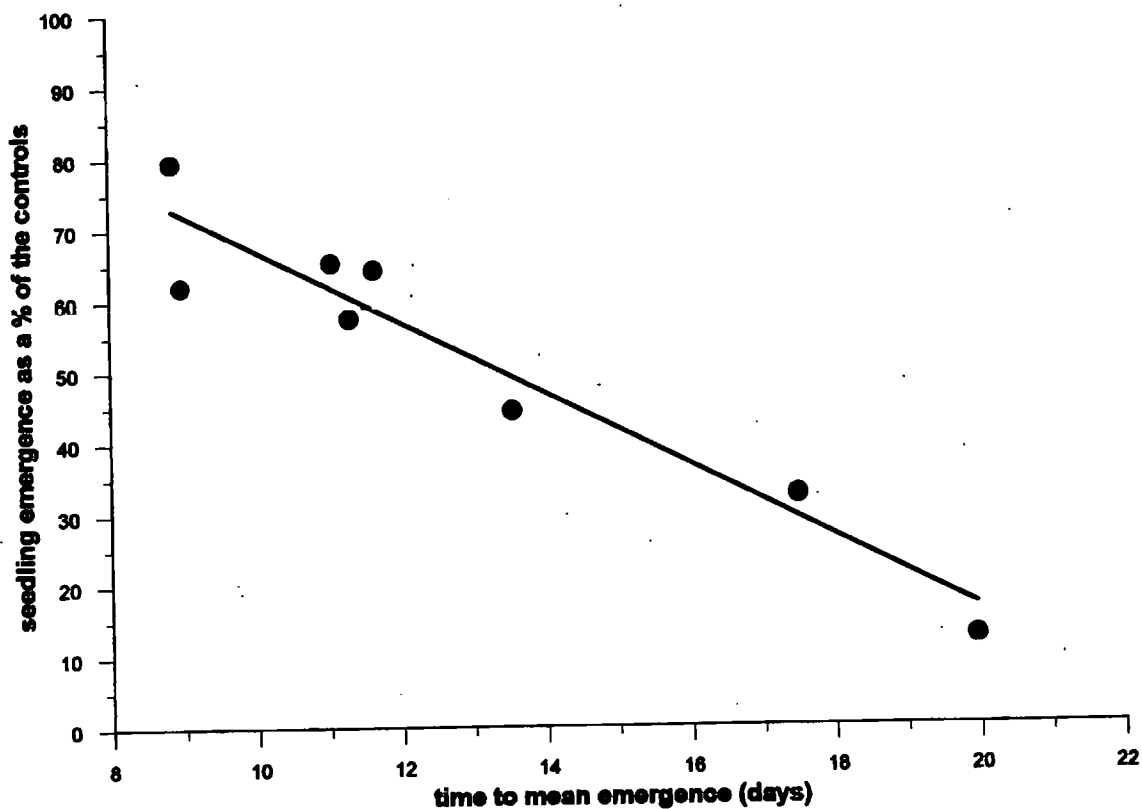


Figure 14 The relationship between the time taken for wheat seedlings cv. Mercia (lot No. 14, see Table 3) inoculated with *Microdochium nivale* macroconidia to emerge and the final number that emerged expressed as a percentage of the controls ($y = -5.046x + 117.5$; $R^2 = 0.92$; $P < 0.01$).

and averaged 97.5%. However, both temperature and soil water did have a significant effect on the emergence of wheat seed naturally contaminated by *M. nivale* ($P < 0.001$). As with artificially inoculated seed, the final emergence of contaminated seed decreased with decreasing temperature and soil water (Figure 15). Maximum emergence (91.0%) was seen at 12°C and -0.005 MPa and minimum emergence (29.0%) at 6°C and -0.108 MPa. Statistical analysis of the data using analysis of variance did not reveal any evidence of an interaction between temperature and soil water with respect to seedling emergence ($P = 0.489$). There is therefore good evidence from this investigation that temperature and soil water act independently on the pre-emergence death of seedlings.

As in the previous experiment with artificially inoculated seeds, the time taken for seedlings to emerge was strongly affected by temperature and soil water. Seedlings emerged more quickly in warmer wetter soil. Overall the rate of seedling emergence increased from 0.035 days⁻¹ at 6°C and -0.108 MPa to 0.091 days⁻¹ at 12°C and -0.05 MPa (Figure 16). Again, following analysis of the data by ANOVA, no interaction between temperature and soil water was seen. Multiple linear regression analysis showed that there was a significant relationship between temperature and the rate of seedling emergence ($P < 0.001$) and that overall a large proportion of the variation seen in the rate of seedling emergence at the different temperatures and soil water potentials was accounted for by the linear model ($R^2 = 0.88$).

The relationship between the time taken for inoculated seedlings to emerge and

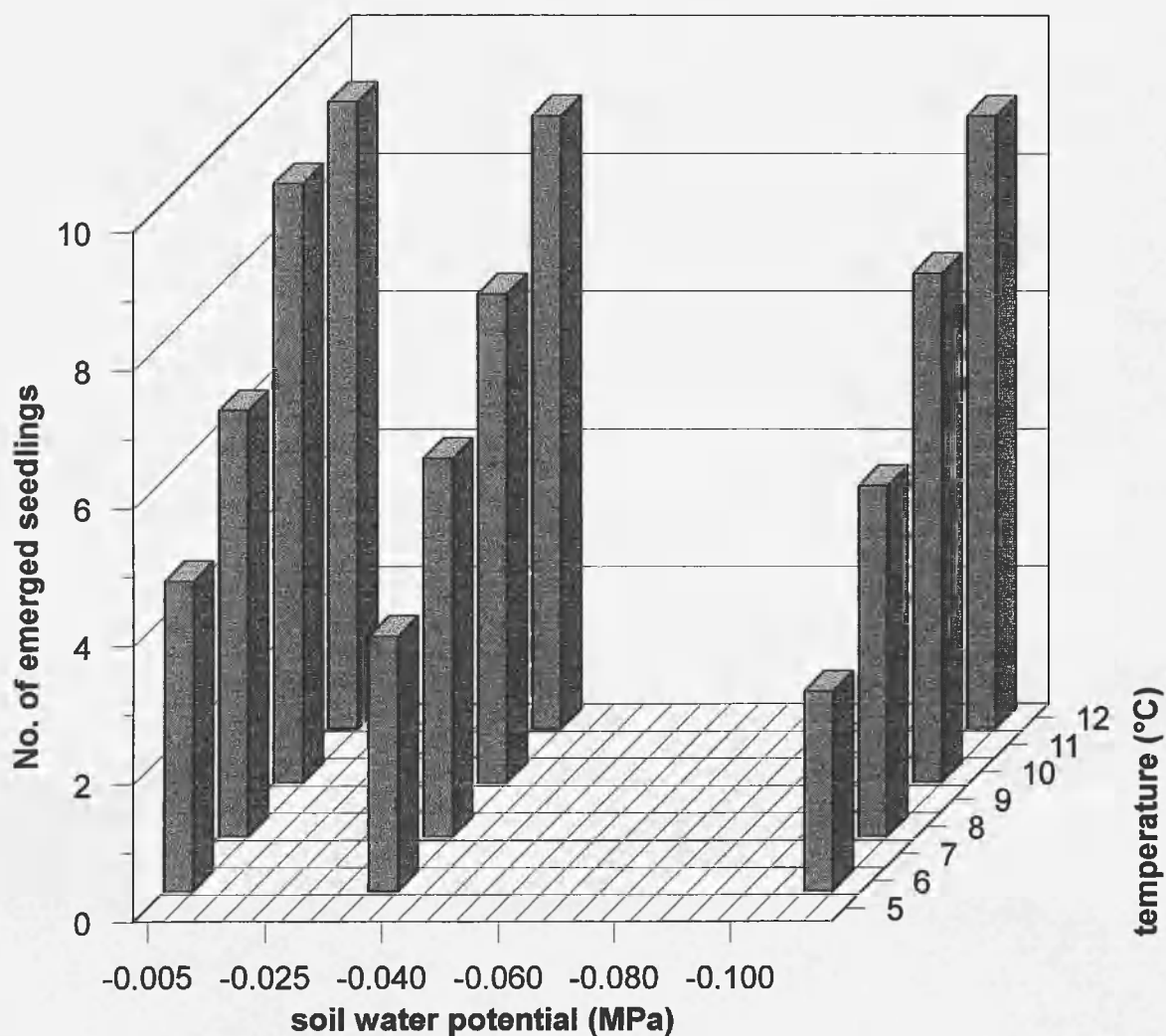


Figure 15 The effect of temperature and soil water potential on the emergence of wheat seedlings (lot No. 15, see Table 3) naturally contaminated with *Microdochium nivale*. LSD ($P = 0.05$) = 0.8; SEM = 0.42; D of F = 108; CV = 20%.

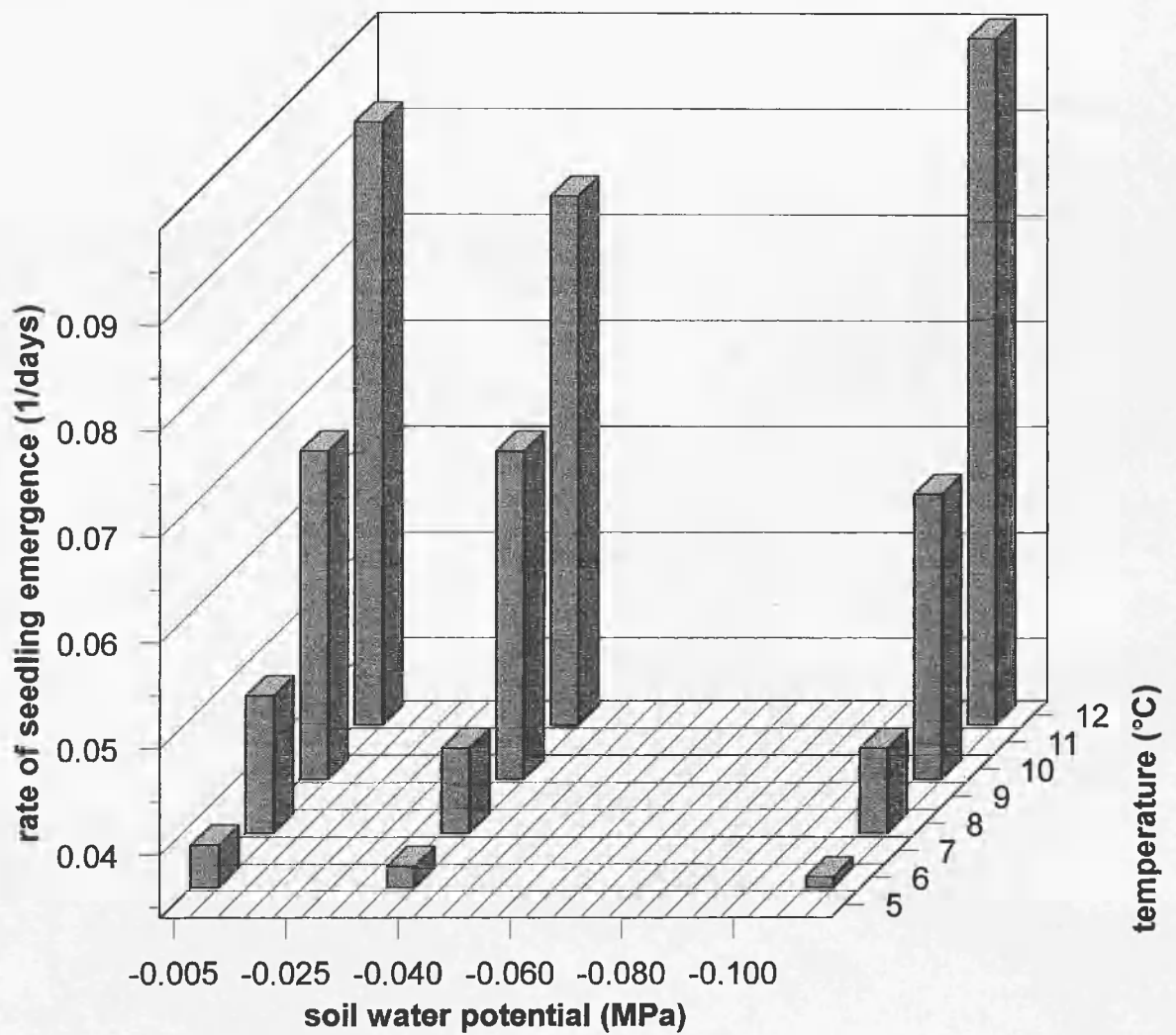


Figure 16 The effect of temperature and soil water potential on the rate of emergence of wheat seedlings (lot No. 15, see Table 3) naturally contaminated with *Microdochium nivale*. LSD ($P = 0.05$) = 0.0038; SEM = 0.00191; D of F = 108; CV = 10.6%.

the final number of seedlings that emerged was again very strong ($R^2 = 95.1\%$). The number of seedlings that emerged decreased significantly ($P < 0.001$) in a linear fashion from 90% to 36% as the time taken for emergence increased from 10 days to 28 days (Figure 17).

DISCUSSION

Previous studies performed in pots in the glasshouse investigating the effect of soil water on *F. culmorum* seedling blight of wheat (Colhoun and Park, 1964, Colhoun *et al.*, 1968 and Cassell and Hering 1982) all used a maximum value of soil water to describe the water status of the soil over the entire duration of the experiments. However, in the present study seedling blight symptoms were demonstrated not to be directly related to maximum ψ_m but to mean ψ_m . This is not surprising as the water status of a soil will not remain constant following watering and therefore, unless some measure of the degree by which it dries between waterings is provided, it is unlikely that maximum ψ_m provides a meaningful measure of soil water.

In the present study mean ψ_m was considered more important than maximum ψ_m and a significant linear relationship between mean ψ_m and pre-emergence death of seedlings following inoculation with *F. culmorum* conidia was observed. This result agrees with the effect of dry soil on seedling blight observed by Colhoun and Park (1964), Colhoun *et al.* (1968) and Cassell and Hering (1982) which was probably not the result of the maximum soil water values they quoted, but of the mean

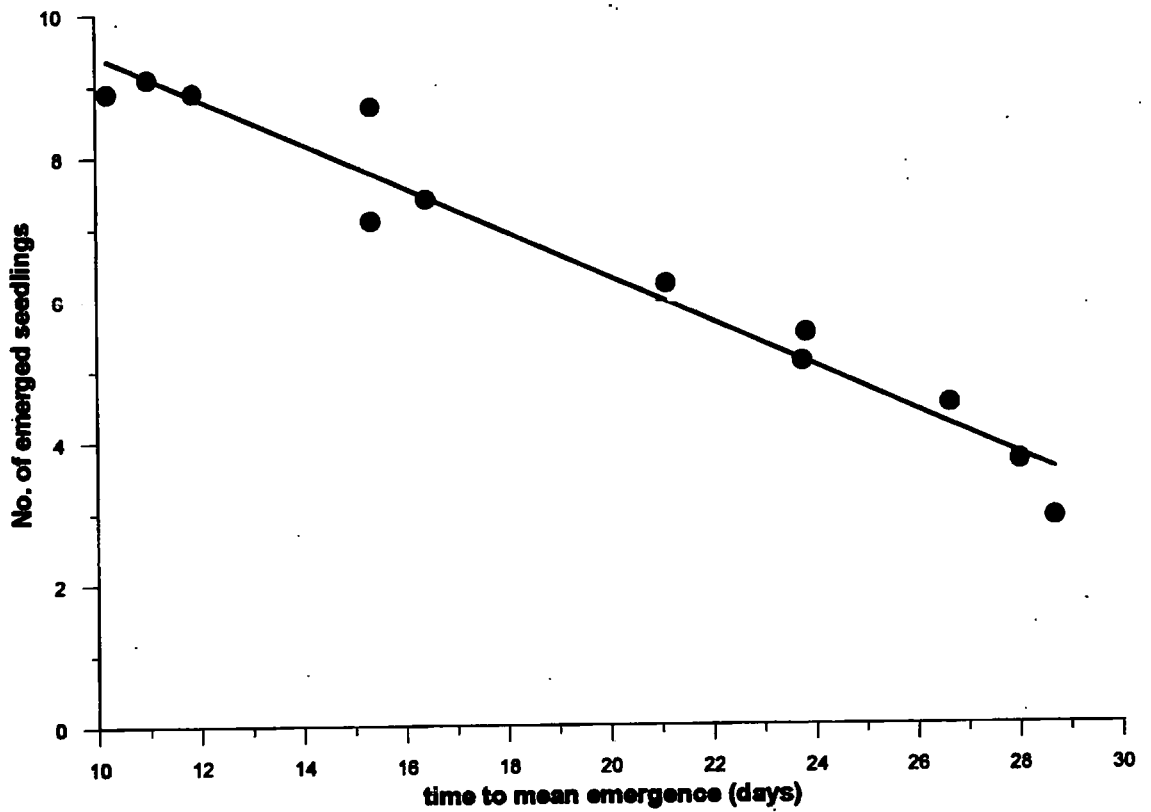


Figure 17 The relationship between the time taken for wheat seedlings cv. Riband (lot No. 15, see Table 3) naturally contaminated with *Microdochium nivale* to emerge and the final number that emerged ($y = -0.313x + 12.57$; $R^2 = 0.95$; $P < 0.01$).

ψ_m values resulting from the different watering regimes they employed. However, as a result of this, direct and precise comparisons with their results were not possible. In the present study the intervals used between watering had no effect on the relationship between mean ψ_m and disease, and it could be concluded that constant soil watering regimes across experiments are unnecessary when using mean ψ_m as a measure of soil water, although extreme watering intervals should be avoided.

If direct comparisons with previous work could have been made, then the differences between previous results and those of this study might still have occurred because of the complex interaction between the host, pathogen, environmental conditions and soil microflora. The presence of the soil microflora in the unsterilised field soils they used may have had a profound effect on their results (Liddell and Burgess, 1988). In addition, the occurrence of soil-borne *F. culmorum* in their soils could also have affected their results such that they may have been studying the effects of the interaction of seed and soil-borne inoculum. As the aim of the present study was to develop a simple reproducible system for use in subsequent studies, the use of unsterilised field soil, which can differ both in textural class and microflora, was avoided. Caution must therefore be taken when applying the results of the present study to a field situation.

The effects of temperature and soil water on seedling blight caused by *M. nivale* were similar whether artificially inoculated or naturally contaminated seed was used. In agreement with previous work with artificially inoculated seed (Millar and Colhoun, 1969) the incidence of pre-emergence seedling death was greatest in cold

dry conditions. The increase in disease severity observed with decreasing temperature and soil water potential was closely correlated to the reduced rate of seedling emergence seen in cold dry soil.

The relationship between the rate of seedling emergence and the severity of seedling blight caused by *F. culmorum* was investigated by Malalasekera and Colhoun (1968) in glasshouse pot experiments. They soaked wheat seeds in water prior to sowing to simulate the increased rate of seedling emergence seen in wet soil. They showed that pre-emergence death was reduced as the rate of seedling emergence increased and concluded that the faster the seedlings emerged the greater their chance of escaping infection. The effect of soaking seeds before sowing on embryo infection by *Pythium ultimum* was investigated by Fukui *et al.* (1994). They showed that soaking wheat seeds prior to sowing decreased the incidence of embryo infection by *Pythium ultimum*. However, they concluded that this reduction in infection was due to the increased loss of seed exudate during soaking and not to the effect of soaking on the rate of seedling emergence.

Despite the fact that the work of Fukui *et al.* (1994) casts some doubt on that of Malalasekera and Colhoun (1968) it appears from the present study that the rate of seedling emergence, which was strongly affected by temperature and ψ_m , does indeed have a significant effect on subsequent seedling blight symptoms. In the present study close linear relationships were seen between the time taken for seedlings to emerge and the final number of emergent seedlings, for seed either artificially inoculated with or naturally contaminated by *M. nivale*. This suggests that

the rate of seedling emergence determined the severity of seedling blight disease symptoms seen and not the specific temperature or soil water values. However, different linear relationships for final seedling emergence and the time taken for seedlings to emerge were seen with respect to artificially inoculated and naturally contaminated seed. This result shows that besides factors affecting the rate of seedling emergence, other factors such as inoculum load may play an important role in determining the severity of disease symptoms. The effect of inoculum load on the severity of disease symptoms from seed naturally contaminated by *M. nivale* is not well documented and will be investigated in the following chapter of this thesis.

CHAPTER 6

The relationship between temperature and natural *Microdochium nivale* contamination of winter wheat seed and the effect on subsequent seedling emergence and seedling growth.

INTRODUCTION

The effect of temperature on the subsequent severity of *M. nivale* seedling blight of wheat seedlings following artificial inoculation of seeds with conidia was investigated in glasshouse pot experiments by Millar and Colhoun (1969). These workers showed that the severity of disease was most severe in cold (6.1°C) soil. In addition, they examined the effect of increasing inoculum load on disease severity and showed that as the number of spores per seed increased from 1×10^{-1} to 1×10^6 so did disease, as indicated by an amended disease index which increased from 100 to 300 respectively.

The effect of temperature on the emergence of wheat seedlings following artificial inoculation with *M. nivale* conidia was studied in Chapter 5 and, in agreement with the work of Millar and Colhoun (1969), most severe disease symptoms were seen in cold soil. The effect of temperature on the pre-emergence death of wheat seedlings naturally contaminated by *M. nivale* was also studied in the previous chapter and, similarly to artificially inoculated seed, the greatest number of seedlings died before emerging, in cold conditions.

It is known that under field conditions the severity of pre-emergence seedling death is affected by the percentage of naturally contaminated seeds in a given batch (Humphreys *et al.*, 1995). The greater the percentage of contaminated seeds the greater the likelihood of pre-emergence seedling death. However, the relationship between temperature and inoculum load, with respect to naturally contaminated seed

and its effect on seedling emergence has not been thoroughly investigated.

The aim of the work in this chapter was twofold: (i) to investigate the relationship between the percentage of seeds contaminated by *M. nivale*, temperature and pre-emergence seedling death and subsequent seedling growth, and (ii) to investigate the relationship between *M. nivale* contamination of wheat seed and wheat seed vigour, as measured by the rate of seedling emergence.

MATERIALS AND METHODS

Sowing of seed

Winter wheat seed cv. Riband (seed lot Nos 15, 17, 18, 19, 20, 21 (Table 3)) was used in this study. Isolations from wheat seeds were taken to determine whether natural seed-borne *M. nivale* contamination was present. Four hundred seeds were placed, five to a Petri dish, onto PDA and incubated at 10°C. After 14 days the fungal colonies present were identified from their colony and spore morphology.

The germination potential of the seed was determined by placing seed treated with the fungicide guazatine (600 µg of ai g⁻¹ of seed) (Rennie and Gorey, 1988 and Cockerell, 1995) onto moist filter paper in Petri dishes at four different temperatures. The seeds were incubated at 6°C, 8°C, 10°C and 12°C until germination had occurred and the number of germinated seeds was recorded daily. Owing to the adverse effect of *M. nivale* on the normal development of seedlings, seed germination was assumed

if any evidence of viability was seen prior to disease symptoms developing. Five Petri dishes each containing 50 seeds were used for each seed lot at each temperature.

Seeds were then sown into compost in glass jars and placed in one of four constant temperatures. For each of the six seed batches used, seeds were sown at the rate of 10 seeds per glass jar into 60 g of sterilized soil-based compost (John Innes Seed) to a depth of 2 cm (Table 4). Five replicate jars were used for each seed batch at each temperature. The jars were then placed in an incubator set at either 6°C, 8°C, 10°C or 12°C according to a randomised block design, and illuminated for 12 hours per day by Philips cool-white fluorescent tubes.

Assessments of seedling emergence and seedling growth

The total number of emerged seedlings in each jar was counted daily until no further seedlings emerged on five successive days. After this time no subsequent emergence was seen in any jar. The mean seedling emergence time in days was calculated using Equation 1 as previously described (Khah *et al.*, 1986) (Chapter 2). The mean rate of emergence was calculated as the reciprocal of the mean seedling emergence time in days .

Following complete emergence, the length of each seedling's coleoptile and longest root was measured.

Data analysis

Data were analysed statistically using factorial ANOVA with *M. nivale* contamination and temperature as factors, and seedling emergence, coleoptile length and root length as variables. Data for the rate of seedling emergence were modelled with the percentage of *M. nivale* contamination in each seed batch and temperature using parallel regression analysis to determine if *M. nivale* contamination affected seed vigour as measured by the rate of seedling emergence. Statistical analyses were performed on a personal computer using the software program Genstat 5 (Lawes Agricultural Trust, Rothamsted Experimental station). Student's "t" tests were performed by hand using a pocket calculator.

RESULTS

(i) The Relationship between the Percentage of Seeds Contaminated by *M. nivale*, Temperature and the Pre-emergence Death of Seedlings and Subsequent Seedling Growth

***Microdochium nivale* seed contamination and germination potential**

The percentage contamination of seeds with *M. nivale* was found to be 4%, 8%, 19%, 40%, 44% and 72% for seed lots Nos 21, 20, 19, 18, 17 and 15 (Table 3) respectively. The germination potential of the seed batches was good, and better than the minimum standard for certified seed (85%) at each of the temperatures tested

(Table 16).

Seedling emergence

Temperature and natural *M. nivale* seed contamination had significant effects on seedling emergence ($P < 0.01$). Seedling emergence decreased with decreasing temperature and with increasing percentage *M. nivale* contamination (Figure 18a and 18b). Mean seedling emergence was highest from seed lots No. 20 and 21, 9.5 and 9.4 seedlings respectively, and lowest from seed lots No. 15 and 18, 6.2 and 6.1 seedlings respectively.

Table 16 Germination of seed batches contaminated by *Microdochium nivale* at four temperatures. Germination tests were performed on moist filter paper in Petri dishes.

Temperature (°C)	<i>Microdochium nivale</i> contamination (%)					
	4	8	19	40	44	72
12	96	98	98	95	94	91
10	98	94	100	94	96	94
8	97	98	96	98	100	92
6	98	98	99	100	98	90
Mean	97.2	97.0	98.2	96.8	97.0	91.8
SE \pm	0.48	1.00	0.85	1.37	1.29	0.85

Figure 18a

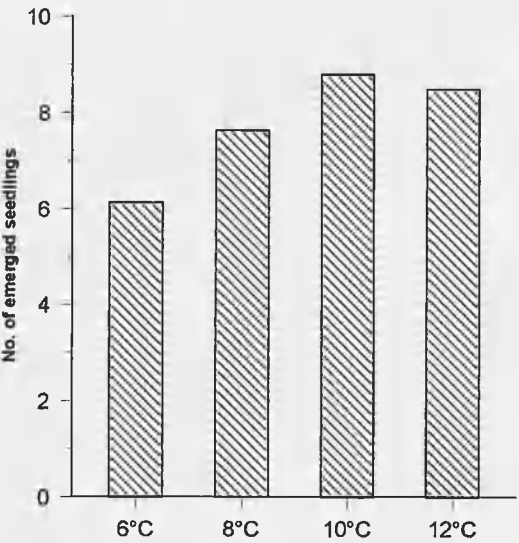


Figure 18b

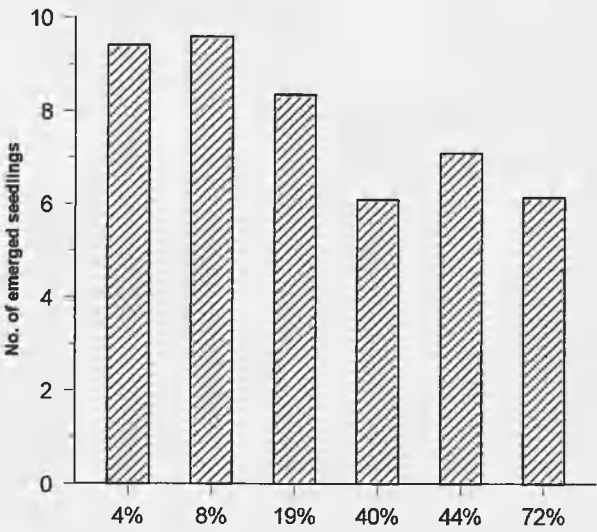


Figure 18c

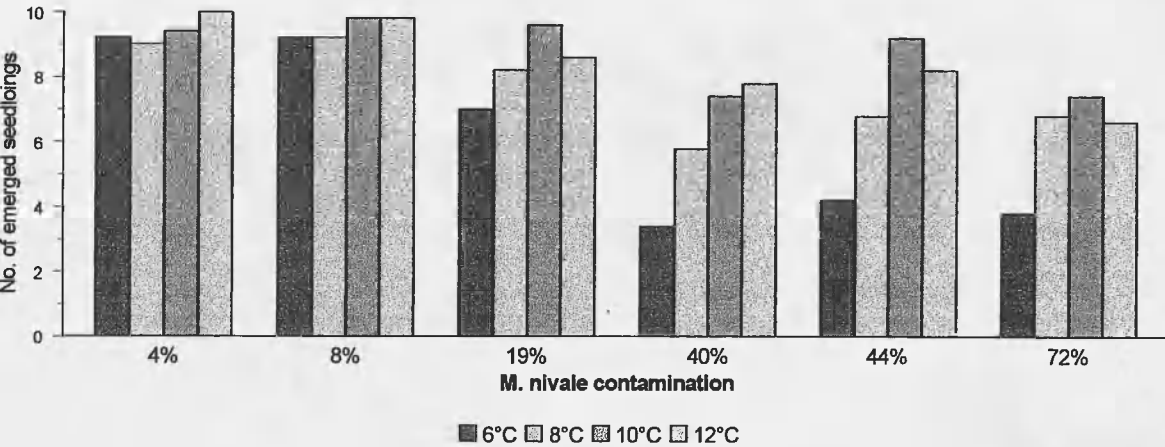


Figure 18 The effect of (a) temperature (SEM = 0.232), (b) *Microdochium nivale* seed contamination (SEM = 2.85) and (c) The relationship between temperature and natural *M. nivale* seed contamination (SEM = 0.57; LSD ($P = 0.05$) = 1.1) on the emergence of wheat seedlings cv Riband (lot No. 21, 20, 19, 18, 17 and 15, see Table 3). D of F = 96; CV = 16%.

The mean number of emerged seedlings (6.1) from seed lot No. 18 (40% *M. nivale*) appeared to be lower than expected with reference to the other seed lots tested. However, there was no significant difference ($P < 0.05$) in seedling emergence between seed lots Nos 18, 17 and 15. A significant interaction was observed between temperature and seed contamination ($P < 0.01$). The reduction in seedling emergence at low temperatures was greatest from seed batches with a large percentage of seeds contaminated by *M. nivale* ($> 40\%$ contamination). As the temperature decreased from 12° C to 6° C seedling emergence decreased by 56% from heavily contaminated seed but by only 8% from seed with few contaminated grains (4%). In addition, the effect of increasing seed contamination on seedling emergence was greatest in cold soil (6° C). Seedling emergence decreased by 44% at 12° C and by 63% at 6° C (Figure 18c).

Coleoptile length

Temperature and *M. nivale* seed contamination had significant effects on the length of coleoptiles of emerged seedlings ($P < 0.01$). Temperature and *M. nivale* seed contamination acted independently on coleoptile length and no significant interaction was observed ($P = 0.05$). Mean coleoptile length was shortest (46.6 mm) in cold soil (6°C) (Figure 19a). Mean coleoptile length was longest (56.6 mm) in seedlings from seed lot No. 15 (4% *M. nivale*) and shortest (44.3 mm) in seedlings from seed lot No. 19 (40% *M. nivale*) (Figure 19b). As with seedling emergence, seed lot No. 18 appeared to produce seedlings with shorter coleoptiles than expected with

Figure 19a

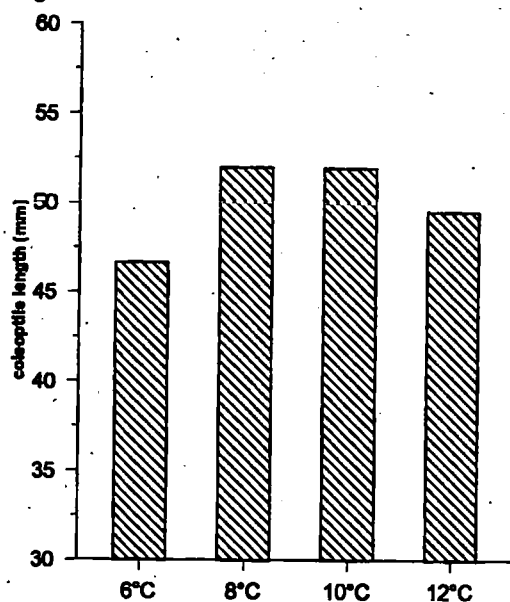


Figure 19b

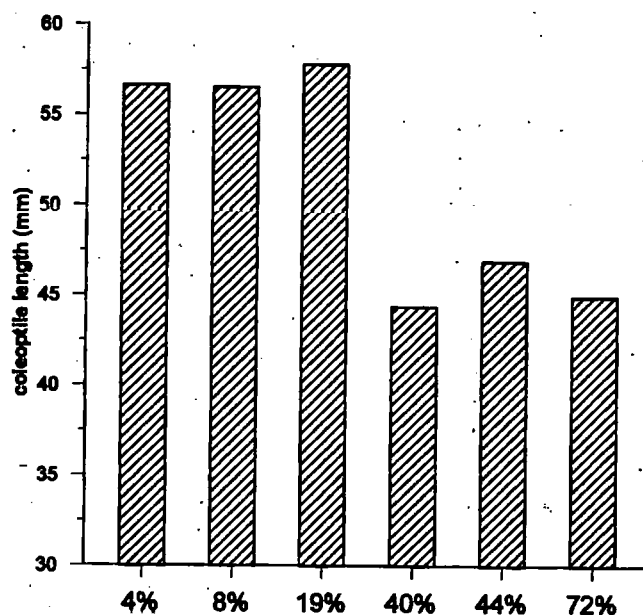


Figure 19 The effect of (a) temperature (LSD ($P = 0.05$) = 2.2; SEM = 1.10) and (b) *Microdochium nivale* seed contamination (LSD ($P = 0.05$) = 2.6; SEM = 1.35) on the coleoptile length of emerged wheat seedlings cv Riband (lot No. 21, 20, 19, 18, 17 and 15, see Table 3), D of F = 96; CV = 12%.

reference to the other seed lots tested although there was no significant difference ($P < 0.05$) in coleoptile length between seed lots Nos 19, 20 and 21.

Root length

Temperature and *M. nivale* seed contamination had significant effects on the length of the longest root of emerged seedlings ($P < 0.001$). Temperature and *M. nivale* seed contamination acted independently on root length and no significant interaction was observed ($P = 0.05$). Mean root length decreased as the temperature decreased from 12° C to 6° C and *M. nivale* seed contamination increased from 4% to 72%. Mean root length was longest 94.1 mm in seedlings at 12° C and shortest 66.9 mm at 6°C (Figure 20a). Mean root length was longest 105.7 mm from seed lot No. 21 (4% *M. nivale*) and shortest 68.3 mm from seed lot No. 15 (72% *M. nivale*) (Figure 20b).

(ii) The Relationship between Natural *M. nivale* Contamination, Temperature and the Rate of Seedling Emergence

Close linear relationships were seen between temperature and the rate of seedling emergence for each of the six seed lots tested (Figure 21). Multiple parallel regression analysis showed that the regression lines fitted to the data were best described ($P < 0.01$) by a model where each regression line had a separate slope and

Figure 20a

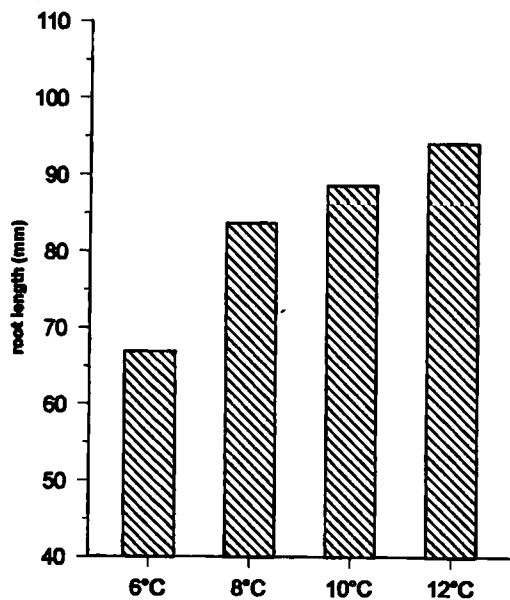


Figure 20b

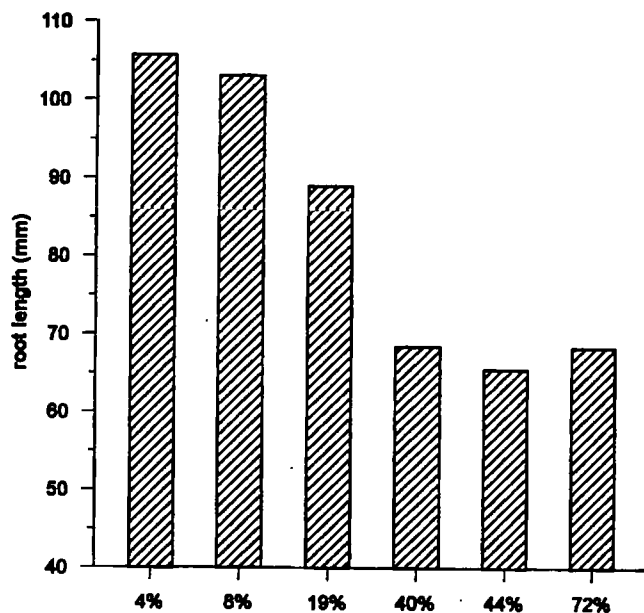
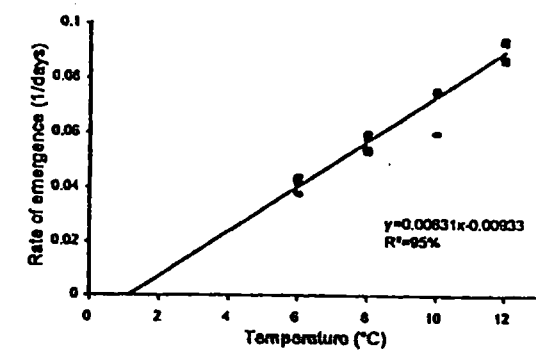
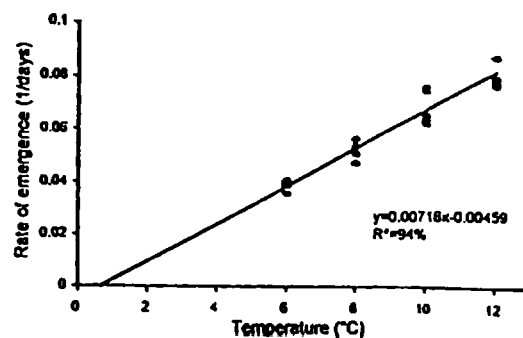


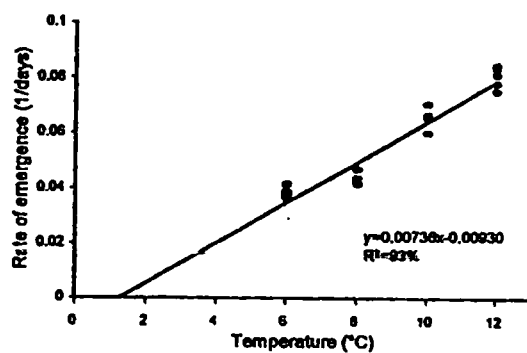
Figure 20 The effect of (a) temperature (LSD ($P = 0.05$) = 5.0; SEM = 2.56) and (b) *Microdochium nivale* seed contamination (LSD ($P = 0.05$) = 6.2; SEM = 3.14) on the root length of emerged wheat seedlings cv Riband (lot No. 21, 20, 19, 18, 17 and 15, see Table 3). D of F = 96; CV = 17%.



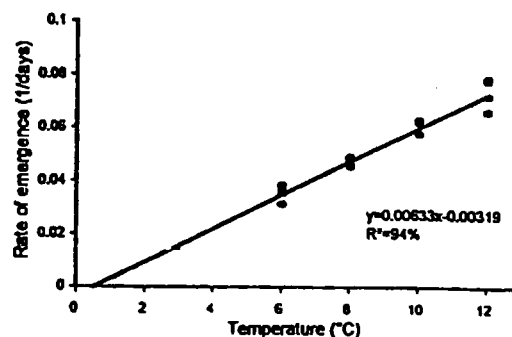
• M. nivale contamination (4%)



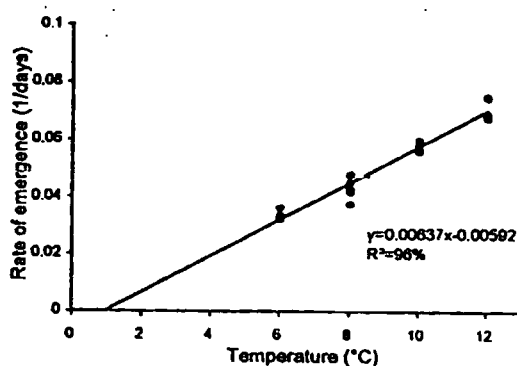
• M. nivale contamination (8%)



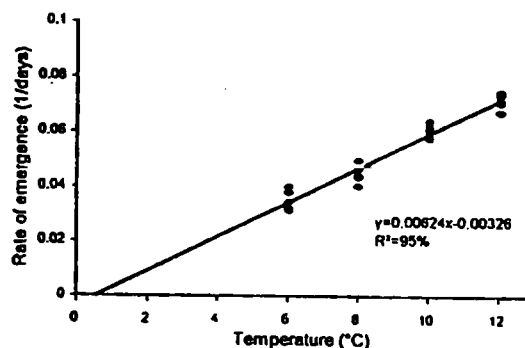
• M. nivale contamination (19%)



• M. nivale contamination (40%)



• M. nivale contamination (44%)



• M. nivale contamination (70%)

Figure 21 The relationship between temperature and wheat seedling emergence for six seed lots of winter wheat cv. Riband (lot No. 21, 20, 19, 18, 17 and 15, see Table 3) naturally contaminated by *Microdochium nivale*.

intercept.

Statistical examination of the variance covariance matrix of the model parameter estimates for each of the six seed lots tested using “t” tests showed there to be no significant difference ($P = 0.05$) between individual intercepts, but significant differences were observed between slopes ($P < 0.001$). The base temperature for emergence and the thermal time to emergence for each seed lot was estimated from the intercepts and the slopes of each graph respectively (Table 17).

A close relationship ($R^2 = 0.902$) was observed between the thermal time to emergence and the percentage of *M. nivale* contaminated seeds in each lot (Figure 22). The thermal time to emergence increased from 120.4° C at 4% *M. nivale* seed contamination to 160.3° C at 72% *M. nivale* seed contamination.

Seedlings took longer to emerge in cold soil and ultimately fewer seedlings emerged in cold conditions. The relationship between the time taken for seedlings to emerge and the final number of emerged seedlings was however different for each of the seed lots tested. A delay in emergence affected heavily contaminated seed lots more than lightly contaminated ones (Figure 23).

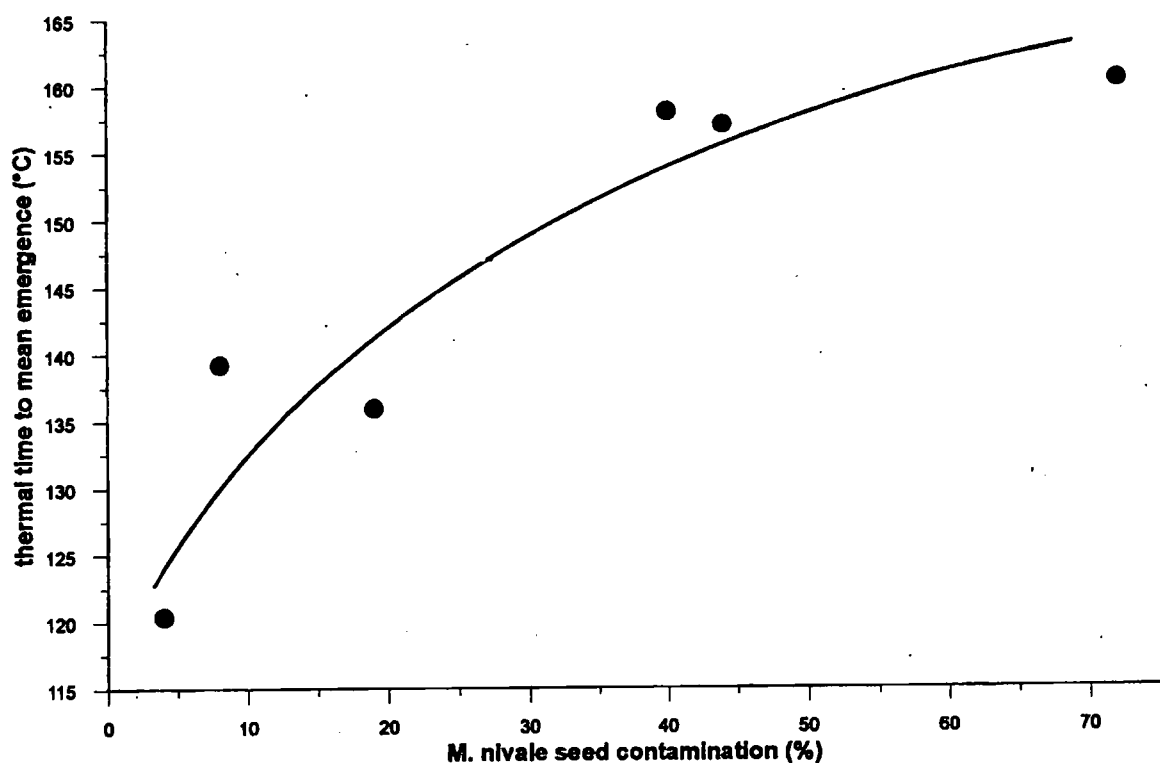


Figure 22 The relationship between the percentage of wheat seeds cv. Riband (lot Nos 21, 19, 18, 17 and 15, see Table 3) naturally contaminated with *Microdochium nivale* and the thermal time to mean seedling emergence ($y = 2.28x^{0.0904}$; $R^2 = 90.2\%$; $P < 0.01$).

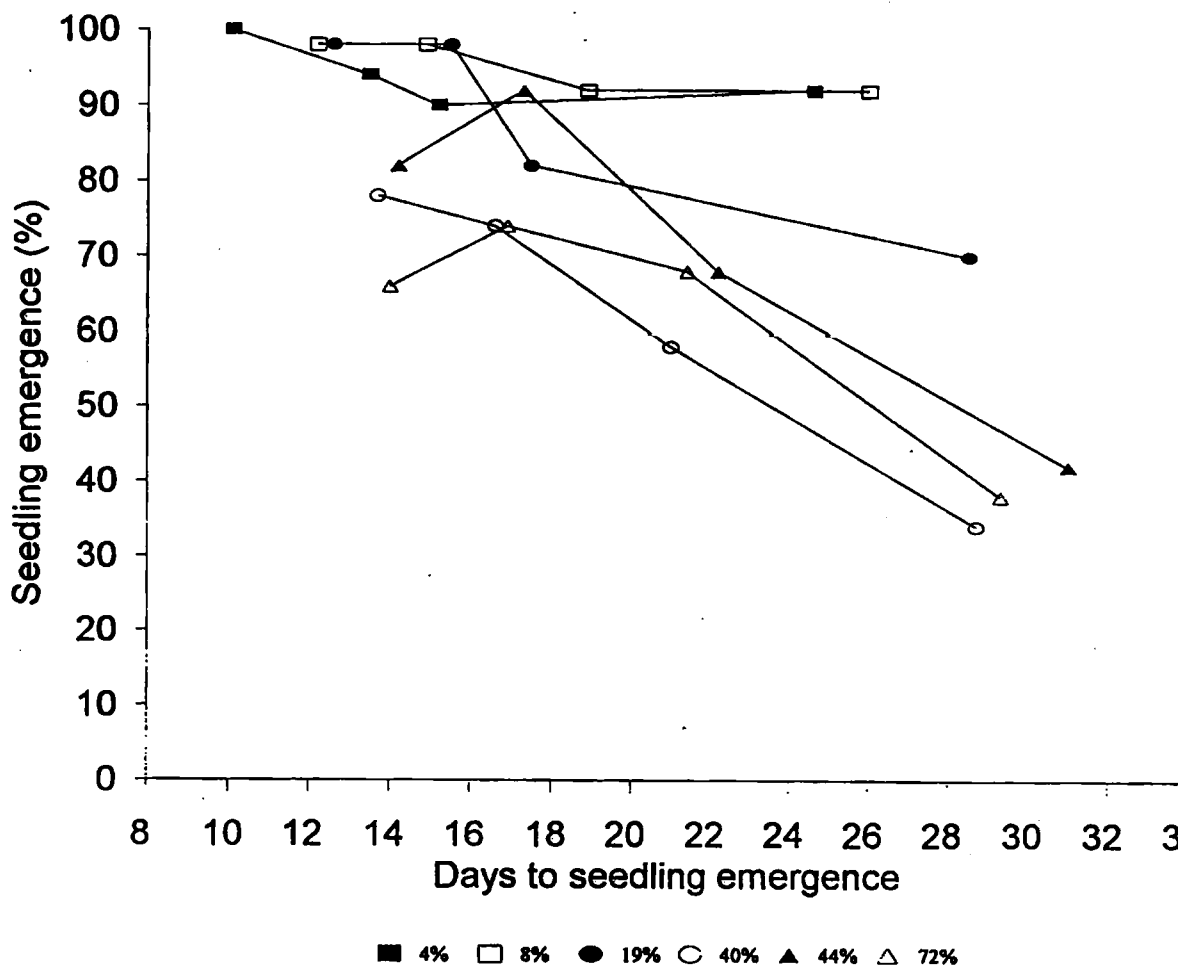


Figure 23 The relationship between the time taken for wheat seedlings to emerge and the final emergence of seedlings cv. Riband (lot No. 21, 20, 19, 18, 17 and 15, see Table 3) naturally contaminated by *Microdochium nivale*.

Table 17 Base temperature for emergence and thermal time to emergence for six seed lots of winter wheat cv. Riband naturally contaminated by *Microdochium nivale* (Table 3)

Seed lot No.	<i>M. nivale</i> (%)	Base Temperature for emergence (t_b) (°C)*	Thermal time to emergence (θ) (°C)*
21	4	1.12	120.4
20	8	0.64	139.2
19	19	1.23	135.9
18	40	0.50	158.0
17	44	0.93	157.0
15	72	0.52	160.3

* $t_b = -a/b$; * $\theta = 1/b$ where $y = a + bx$

DISCUSSION

Work in glasshouse pot experiments by Millar and Colhoun (1969) showed that an increase in the number of *M. nivale* conidia applied to the surface of wheat seeds prior to planting resulted in a subsequent increase in seedling blight disease severity. A similar result was observed by Humphreys *et al.* (1995) working with naturally contaminated wheat seed in the glasshouse and the field. Their results showed that there was a close linear relationship between the percentage of *M. nivale*

contaminated seeds in a seed lot and the final emergence of seedlings. However, the results presented in this chapter show that the relationship between the percentage of *M. nivale* contaminated seeds and the final emergence of seedlings is not that simple. In general, seedling emergence was seen to decline at a given temperature as the percentage of contaminated seeds increased, although the relationship between *M. nivale* contamination and seedling emergence did not appear to be linear (Figure 18c). For example, more pre-emergence seedling death than expected from the amount of *M. nivale* contamination present was seen from seed lot No. 18. The occurrence of unexpected seedling blight disease severity in the field has been observed and although some of these occurrences may have been due to poor germination potential there is no evidence that this is the case in this study (Table 16).

The percentage of contaminated seed in a seed lot appears to give a good indication as to the potential for disease but it does not account for different inoculum loadings on individual seeds. Millar and Colhoun (1969) showed that, on individual wheat seed, *M. nivale* inoculum load had a significant effect on subsequent disease severity and therefore, in situations where individual seeds in a seed lot are more or less heavily contaminated, unexpected disease severities may occur. Little work has been done on the quantification of *M. nivale* inoculum on individual wheat seeds. Malalasekera and Colhoun (1969) developed a technique to quantify the amount of *F. culmorum* inoculum on wheat seeds by applying conidia to the surface of the seed and then measuring the resultant colony diameter after incubation on agar. They observed a good linear relationship between individual seed spore load and colony

diameter, but were unable to apply this technique successfully to naturally contaminated seed owing to the variations in colony diameter they observed. This result in itself suggests that individual seeds within a seed lot may carry different inoculum loads. For further investigation of the relationship between inoculum load and subsequent disease severity the adoption of a quantitative molecular technique such as competitive PCR may prove useful.

Environmental factors such as temperature and soil water can also affect the severity of disease from naturally contaminated seed (Chapter 5). Indeed the effect of temperature on disease severity was again shown in the work in this chapter. In addition, the results of the present study show a significant interaction between seed contamination and temperature with respect to final seedling emergence. The effect of seed contamination on subsequent seedling emergence was more severe in cold soil and this response was most pronounced when the percentage of contaminated seeds was high. The severity of disease symptoms seen from naturally contaminated seed was therefore a product of inoculum load and temperature. This result may in part explain the findings of Noon and Jackson (1992) who observed different numbers of seedlings emerging from non-fungicide treated plots in field trials in Warwickshire and Lincolnshire. The seed used was heavily contaminated by *M. nivale* (72%) and was treated similarly at both sites. They speculated that the difference in the number of emerged seedlings observed at the two sites was due to the environmental factors; temperature and soil water, although these environmental factors were not closely monitored. It is clear therefore that in addition to *M. nivale* contamination,

temperature will have a significant effect on the severity of the disease and subsequent seedling emergence.

Seedling growth following emergence, as measured by coleoptile length and root length, was also affected by the percentage of *M. nivale* contaminated seed and temperature, although there was no evidence of an interaction between them. The effect of *M. nivale* on seedling growth subsequent to emergence did not appear to be greater under cold conditions.

Observations on the rate of seedling emergence showed that seedlings emerged more slowly in cold soil and that seedlings from seed lots heavily contaminated by *M. nivale* also emerged more slowly. Khah *et al.* (1986) suggested that the thermal time for emergence of wheat seedlings grown in field plots was strongly affected by laboratory germination. However, the different thermal times for emergence of the seed batches tested in this study cannot be fully explained with reference to their germination potential alone. The close relationship between the percentage of *M. nivale* contaminated seeds and the thermal time for emergence of each seed lot suggests that seed contamination may have a significant effect on the rate of seedling emergence. As the rate of seedling emergence is considered to be a measure of seedling vigour (AOSA, 1983) it could be concluded that contamination of wheat seeds by *M. nivale* may reduce seedling vigour. This assumption is reinforced by the interaction observed between temperature and *M. nivale* contamination with respect to pre-emergence seedling death, where the number of non-emergent seedlings became progressively greater in cold conditions with heavily contaminated seed lots.

Conclusive evidence to support the theory that *M. nivale* contamination reduced the vigour of wheat seedlings would be difficult to obtain owing to the many host, pathogen, environment interactions which can occur during seedling emergence. The effect of increasing inoculum load, using artificial inoculum, on the rate of seedling emergence could be investigated, although it is not clear whether conidia on the surface of the seed would produce a similar effect to sub-epidermal mycelium which is often associated with fungal contamination of wheat seeds (Elekes, 1983). Another approach would be to treat naturally contaminated seed with a range of rates of fungicide in an attempt to manipulate the growth of the pathogen. This approach may work as long as pathogenicity is closely correlated with fungicide efficacy. The effect of the fungicide seed treatment on seedling growth would also have to be considered.

CHAPTER 7

Seed-borne *Microdochium nivale* var. *nivale* and *Microdochium nivale* var. *majus*; their occurrence on wheat seed and pathogenicity towards wheat seedlings

INTRODUCTION

Within *M. nivale* there are two distinct varieties, *M. nivale* var. *nivale* and *M. nivale* var. *majus*. These two varieties were first distinguished by Wollenweber (1931) when *M. nivale* was still considered to be *F. nivale*. Wollenweber distinguished between the two varieties on the basis of conidial morphology: *Microdochium nivale* var. *majus* having larger conidia than *M. nivale* var. *nivale*. Gerlach and Nirenberg (1982) defined the varieties further, stating that *M. nivale* var. *nivale* was mostly 1-3 septate whilst *M. nivale* var. *majus* was mostly 3 septate with wider spores. However, Nelson *et al.* (1983) and Litschko and Burpee (1987) did not differentiate any distinct types using these morphological characteristics. More recently Lees *et al.* (1995) used random amplified polymorphic DNAs (RAPDs) to investigate variability within *M. nivale* isolates from wheat in the U.K. This study revealed extensive variability within *M. nivale* and also demonstrated the presence of a uniform sub-group which correlated to *M. nivale* var. *majus* as determined by conidial morphology. A uniform sub-group correlated to *M. nivale* var. *majus* was also confirmed among isolates of *M. nivale* from elsewhere in Europe by isozyme analysis and the study of polymorphisms within the internal transcribed spacer (ITS) region of rDNA (Maurin *et al.*, 1995). In the absence of any further information about the remaining diverse sub-group it was termed *M. nivale* var. *nivale* to distinguish it from the homogeneous *M. nivale* var. *majus* sub-group.

Little is known about the relative occurrence of *M. nivale* var. *nivale* and *M.*

nivale var. *majus* on naturally contaminated winter wheat seed. However, all of the eight isolates of *M. nivale* isolated from wheat seed in the U.K. by Lees *et al.* (1995) were classified as *M. nivale* var. *majus*, on the basis of RAPD analysis and conidial morphology.

Differences in the pathogenicity of *M. nivale* isolates towards cereal seedlings was observed by Perry and Al-Hashimi (1983). They reported large differences in the severity of *Fusarium* seedling blight symptoms on barley seedlings grown in soil in pots, following inoculation of the seed with conidia. Maurin (1993) also observed differences in pathogenicity between *M. nivale* isolates and was able to distinguish differences between *M. nivale* var. *nivale* and *M. nivale* var. *majus*. Isolates of *M. nivale* var. *nivale* were found to be strongly pathogenic whilst isolates of *M. nivale* var. *majus* were only weakly pathogenic. Maurin, however, did not state how pathogenicity was tested or which plants were used.

The aim of the work in this chapter was twofold: (i) to investigate the occurrence of *M. nivale* var. *nivale* and *M. nivale* var. *majus* isolates on naturally-contaminated winter wheat seed and (ii) to investigate the pathogenicity towards wheat seedlings of *M. nivale* var. *nivale* and *M. nivale* var. *majus* isolates recovered from wheat seed.

MATERIALS AND METHODS

(i) The Occurrence of *M. nivale* var. *nivale* and *M. nivale* var. *maius* on Naturally Contaminated Winter Wheat Seed

Isolates of *M. nivale* were recovered from seven seed lots of wheat seed naturally contaminated by *M. nivale*. The seed lots used were Nos 2, 5, 12, 13, 15, 16, and 17 (Table 3) and were sourced from across the U.K., from Invergowrie in Tayside to Owslebury in Hampshire. However, the majority of the seed lots were from the east of England.

Isolates of *M. nivale* were recovered on PDA from one hundred seeds from each seed lot following treatment with sodium hypochlorite (see Chapter 2). The resultant fungal colonies were identified on the basis of their colony and spore morphology. Isolates of *M. nivale* were subcultured on PDA and incubated at 15°C for 10 days. These uncontaminated *M. nivale* isolates were then freeze-dried and stored in glass ampoules or in liquid nitrogen.

Cultures of *M. nivale* were grown on PDA at 15°C for 15 days in the dark and then for a further 10 days with light to induce sporulation (see Chapter 2). Sporodochia were removed from the cultures and the spores suspended in 500 µl of sterile distilled water. Aliquots (100 µl) of the spore suspension were spread over tap water agar in 9 cm Petri dishes and incubated at 15°C. After 24 hours single germinated conidia were located using a low powered microscope (x40) and

transferred to separate Petri dishes containing 15 ml of PDA.

Fourteen cultures from each seed lot (10 cultures for lot No. 2) were selected at random and further classified to the varieties *majus* or *nivale* by D. W. Parry and H. N. Rezanoor using primers designed for the internal transcribed spacer (ITS) region in the polymerase chain reaction (PCR), followed by restriction enzyme digestion of the PCR product (Maurin *et al.*, 1995).

(ii) Pathogenicity of *M. nivale* var. *nivale* and *M. nivale* var. *majus* Isolates towards Wheat Seedlings

Isolates used

The pathogenicity towards winter wheat seedlings cv. Brigadier seed lot No. 4 (Table 3) of four isolates of *M. nivale* var. *nivale* and four isolates of *M. nivale* var. *majus* was investigated. Two isolates of *M. nivale* var. *nivale* and two isolates of *M. nivale* var. *majus* from seed lot No. 2 (isolate No. 1, 2, 3, and 4 respectively) and one isolate of *M. nivale* var. *nivale* and one isolate of *M. nivale* var. *majus* from each of the seed lots Nos 16 (isolate No. 5 and 6) and 12 (isolate No. 7 and 8) were used.

Inoculation of seed

Conidia were produced on PDA and spore suspensions made in sterile distilled water (Chapter 2). Wheat seeds cv. Brigadier were treated with sodium hypochlorite,

washed, dried (Chapter 2) and then inoculated with spores at the rate of 4×10^3 spores per seed by mixing the seed and spore suspension for three minutes in a 250 ml conical flask. Seed used as an uninoculated control was treated similarly, but with sterile distilled water instead of spore suspension.

Seeds were then sown at the rate of 10 seeds per glass jar into 60 g of sterilized compost (John Innes Seed) to a depth of 2 cm (Table 4). Eight replicate jars were used for each inoculated treatment and 16 replicate jars were used for the uninoculated control. The jars were then placed in an incubator set at 6°C and illuminated for 12 hours per day by Philips cool-white fluorescent tubes, according to a randomised block design.

Assessment of seedling emergence

The number of emerged seedlings was counted daily. Seedling emergence was taken to be complete when no further seedlings emerged on five successive days. Seedling emergence was complete 24 days after planting.

Assessment of disease severity on emerged seedlings

The severity of disease on emerged seedlings was assessed 24 days after planting. Seedlings were removed from the compost and examined visually. The seedlings were assigned one of five disease values (0, 1, 2, 3 or 4) according to the severity of the symptoms present (Table 5). Seedling disease values were calculated according to the method previously stated (Chapter 2).

Assessment of seedling growth

Following disease severity assessment, the lengths of each seedling's coleoptile, shoot and longest root were measured.

Data analysis

Data were analysed using ANOVA and differences between treatments were examined using "t" tests. Statistical analysis was performed on a personal computer using the software program Statgraphics and "t" tests were performed by hand using a pocket calculator.

RESULTS

(i) The Occurrence of *M. nivale* var. *nivale* and *M. nivale* var. *majus* on Naturally Contaminated Winter Wheat Seed

Of the 94 isolates examined using the PCR technique 91 gave banding patterns which allowed varietal distinctions to be made. The majority (85) of isolates were classified as *M. nivale* var. *majus* with only six as *M. nivale* var. *nivale*. The *M. nivale* var. *nivale* isolates came from three seed lots: three isolates came from lot No. 2, two came from lot No. 12 and the remaining isolate came from lot No. 16 (Table 18).

Table 18 Isolates of *Microdochium nivale* var. *majus* and var. *nivale** from wheat seed listed according to location.

Lot No.	Location	No. of isolates	
		<i>Majus</i>	<i>nivale</i>
16	Invergowrie, Tayside	13	1
17	Driffield, Yorkshire	13	0
15	Scunthorpe, Humberside	13	0
2	Eriswell, Suffolk	7	3
5	Haverhill, Suffolk	14	0
13	Stowmarket, Suffolk	13	0
12	Owslebury, Hampshire	12	2
Total		85	6

*Classified according to the method of Maurin *et al.* (1995)

(ii) Pathogenicity of *M. nivale* var. *nivale* and *M. nivale* var. *majus* Isolates towards Wheat Seedlings

Seedling emergence

The final emergence of uninoculated seedlings was good (9.8 seedlings). Similar emergence (9.8) was seen from seedlings inoculated with *M. nivale* var. *nivale* conidia. However, significantly fewer ($P < 0.001$) seedlings emerged

subsequent to inoculation with *M. nivale* var. *majus*. The mean emergence for seedlings inoculated with *M. nivale* var. *majus* was 4.9 (Figure 24). Little variation was seen between the emergence of seedlings inoculated with different isolates of *M. nivale* var. *nivale* (9.5 to 10.0), although the emergence of seedlings inoculated with different *M. nivale* var. *majus* isolates varied greatly (0.1 to 8.9).

Disease score on emerged seedlings

No seedling blight disease symptoms were seen on the uninoculated controls (disease severity score 0). However, inoculation with each of the *M. nivale* var. *nivale* and *M. nivale* var. *majus* isolates tested produced disease symptoms on emerged seedlings. Isolates of *M. nivale* var. *majus* produced significantly more ($P < 0.001$) severe disease symptoms (mean disease severity score 50) than isolates of *M. nivale* var. *nivale* (mean disease severity score 19) (Figure 25). Variation in disease severity between pathogen isolates was evident. Disease severity scores for *M. nivale* var. *majus* isolates ranged from 21 to 33 and variation between *M. nivale* var. *majus* isolates ranged from 32 to 98 *M. nivale* var. *majus*.

Seedling growth

There was little statistical evidence ($P < 0.001$) to suggest that inoculation with *M. nivale* var. *nivale* reduced the length of seedlings' coleoptiles or shoots (Figures 26 and 27). However, following inoculation with isolates of var. *nivale* root length was significantly ($P < 0.001$) reduced from 136 mm to 113 mm (Figure 28).

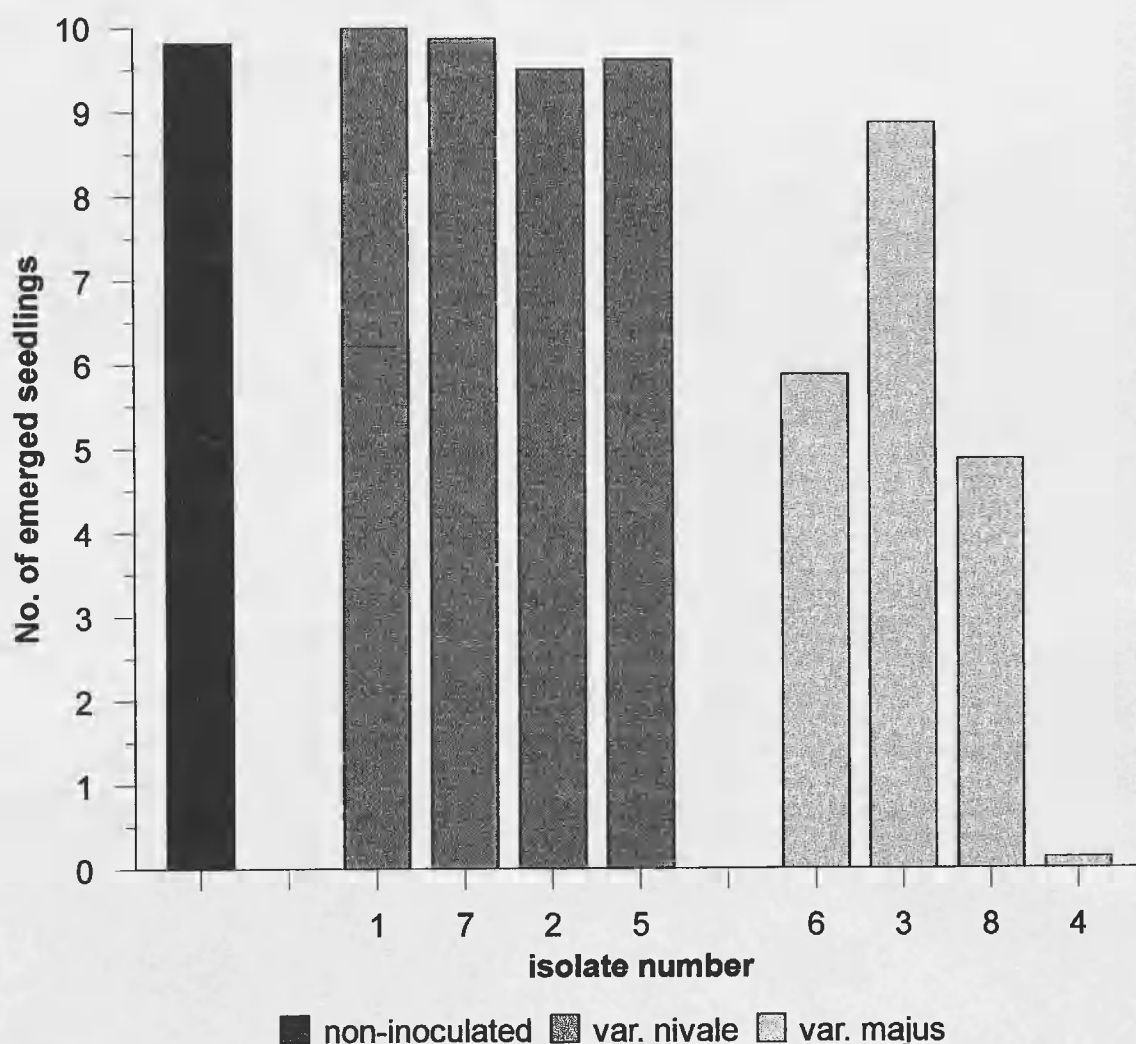


Figure 24 The emergence of wheat seedlings following inoculation of seed (lot No. 4, see Table 3) with conidia from isolates of *Microdochium nivale* var. *majus* and *M. nivale* var. *nivale*. Seedlings were grown at 6°C. The SEM for non-inoculated seed = 0.248 and for inoculated seed = 0.351. The LSD ($P = 0.05$) for non-inoculated vs inoculated = 0.17 and for inoculated vs inoculated = 0.97; D of F = 68; CV = 13%.

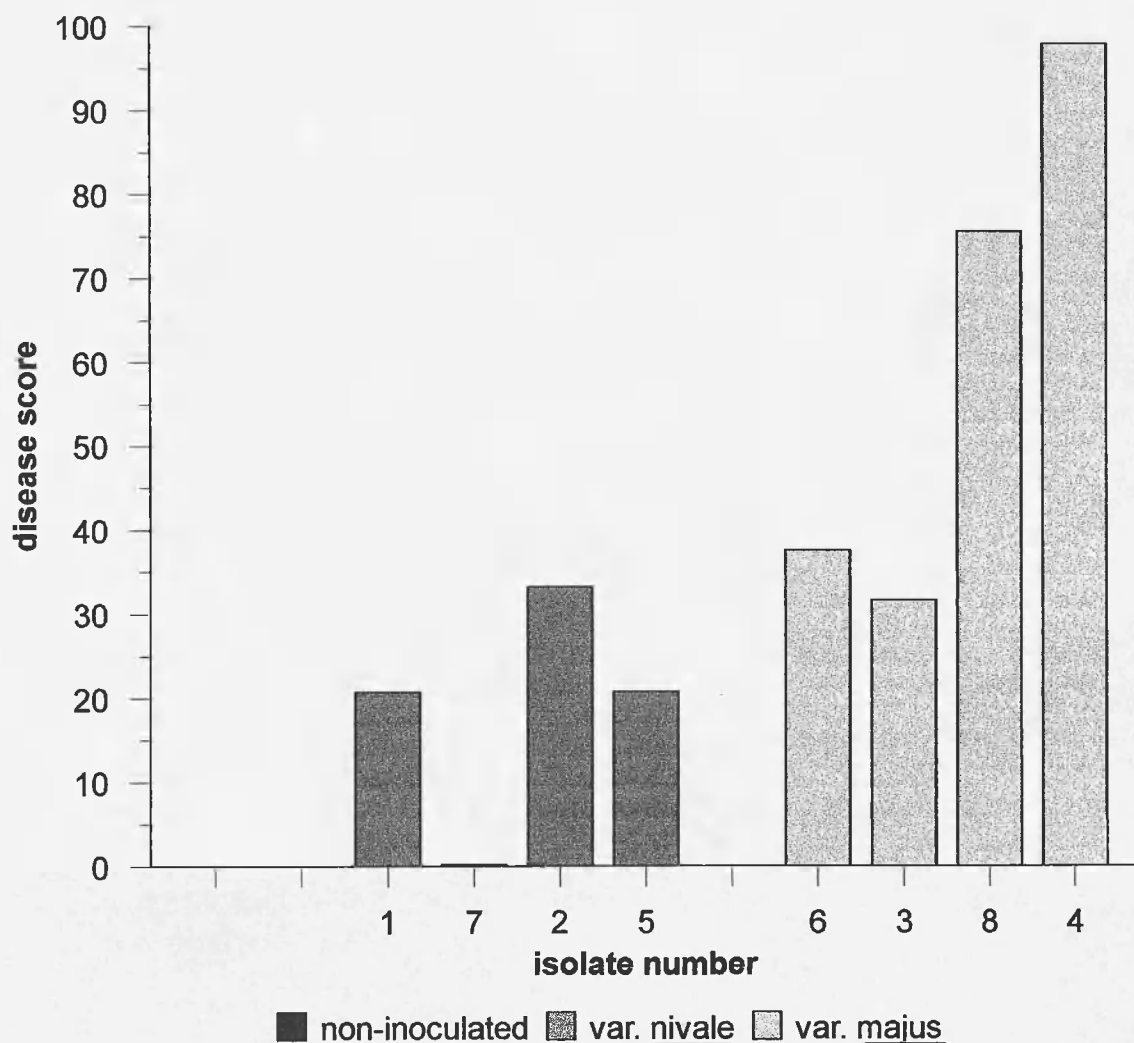


Figure 25 The disease score of emerged wheat seedlings following inoculation of seed (lot No. 4, see Table 3) with conidia from isolates of *Microdochium nivale* var. *majus* and *M. nivale* var. *nivale*. Seedlings were grown at 6°C. The SEM for non-inoculated seed = 1.98 (n = 16), for inoculated seed with the exception of isolate No. 4 = 2.81 (n = 8) and for isolate No. 4 = 7.9 (n = 1); D of F = 61; CV = 22%.

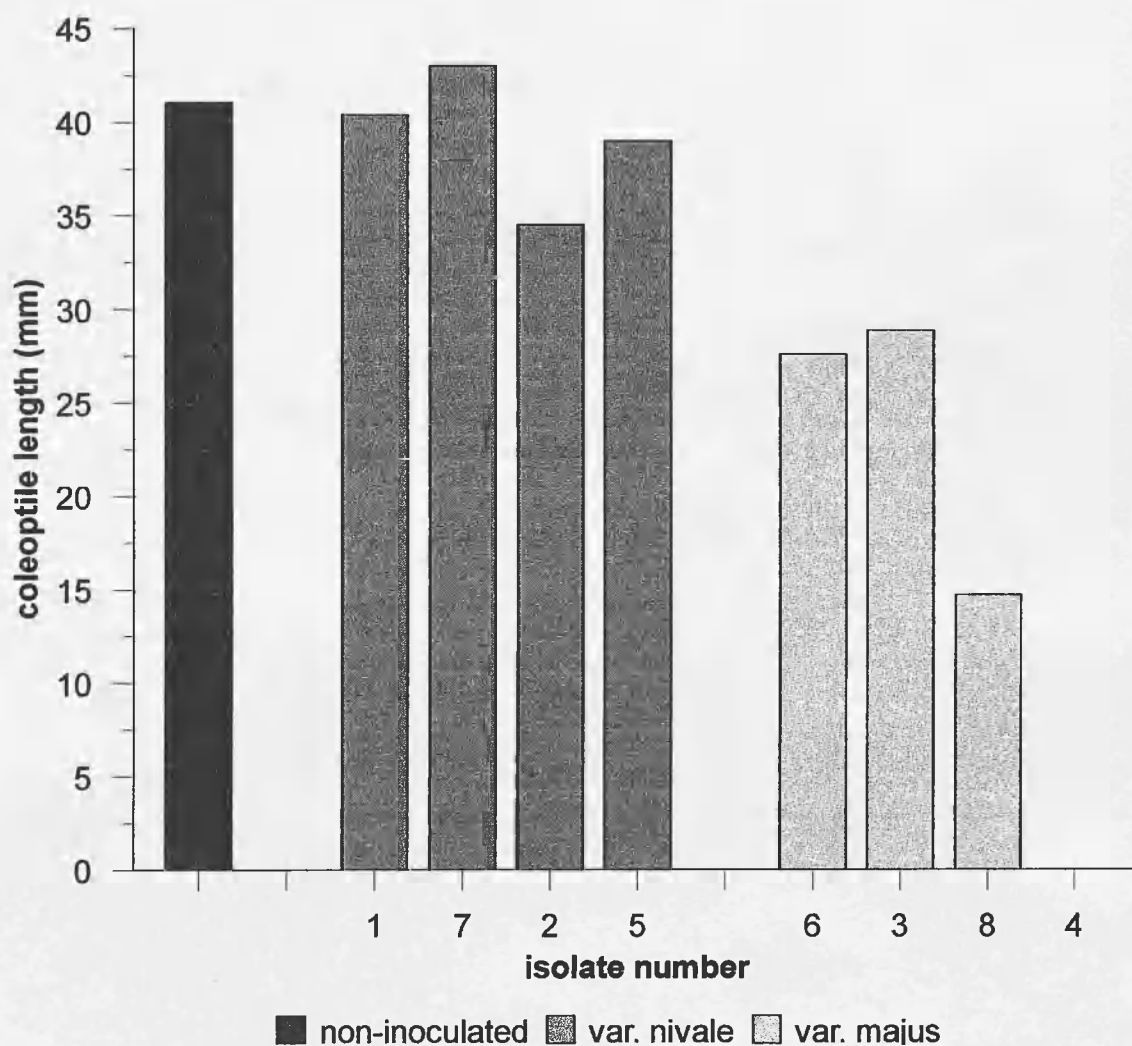


Figure 26 The coleoptile length of wheat seedlings following inoculation of seed (lot No. 4, see Table 3) with conidia from isolates of *Microdochium nivale* var. *majus* and *M. nivale* var. *nivale*. Seedlings were grown at 6°C. The SEM for non-inoculated seed = 0.72 and for inoculated seed = 1.01. The LSD ($P = 0.05$) for non-inoculated vs inoculated = 2.5 and for inoculated vs inoculated = 2.9; D of F = 61; CV = 8.5% .

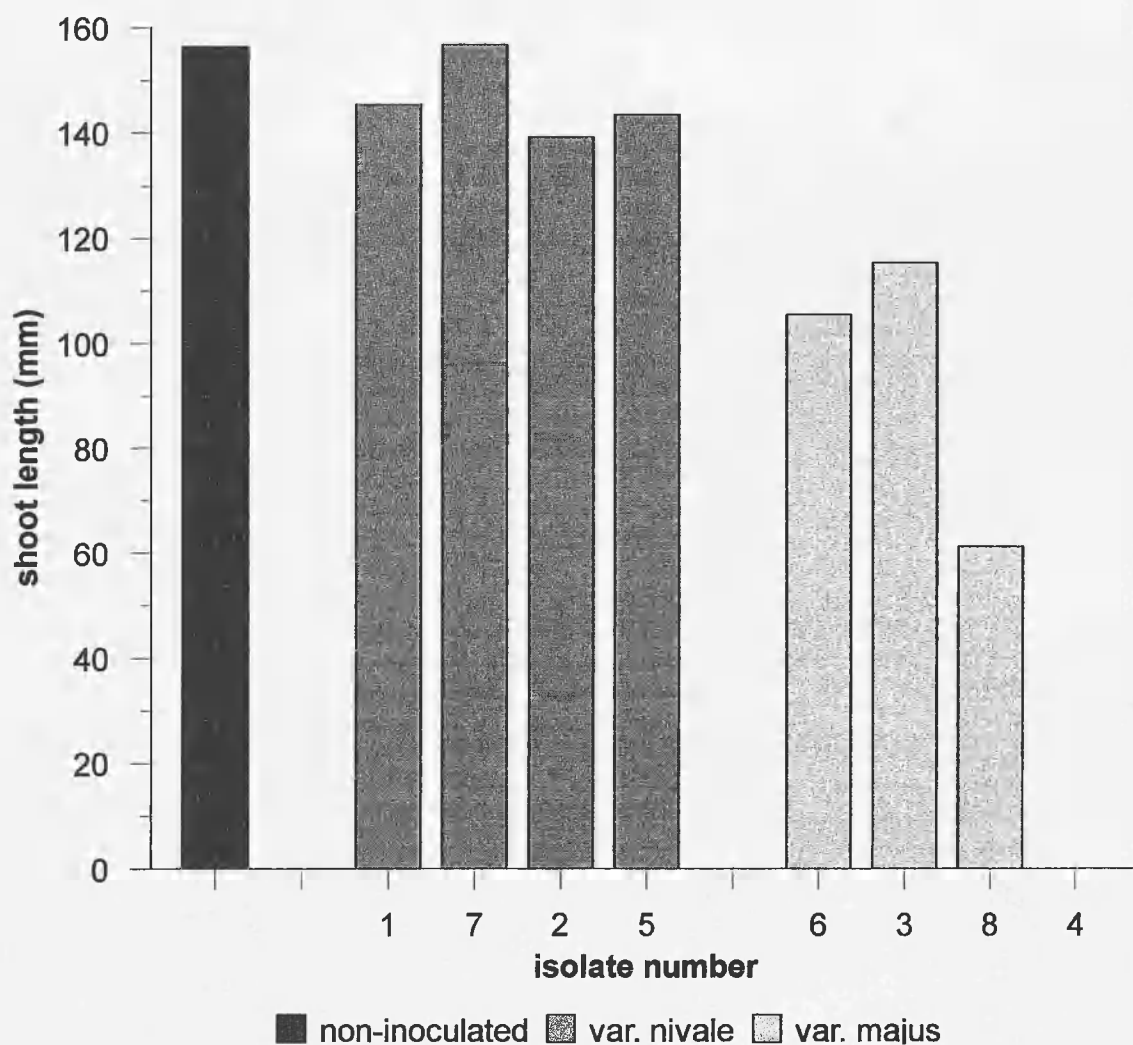


Figure 27 The shoot length of wheat seedlings following inoculation of seed (lot No. 4, see Table 3) with conidia from isolates of *Microdochium nivale* var. *majus* and *M. nivale* var. *nivale*. Seedlings were grown at 6°C. The SEM for non-inoculated seed = 2.76 and for inoculated seed = 3.91. The LSD ($P = 0.05$) for non-inoculated vs inoculated = 9.6 and for inoculated vs inoculated = 11.1; D of F = 61; CV = 8.6% .

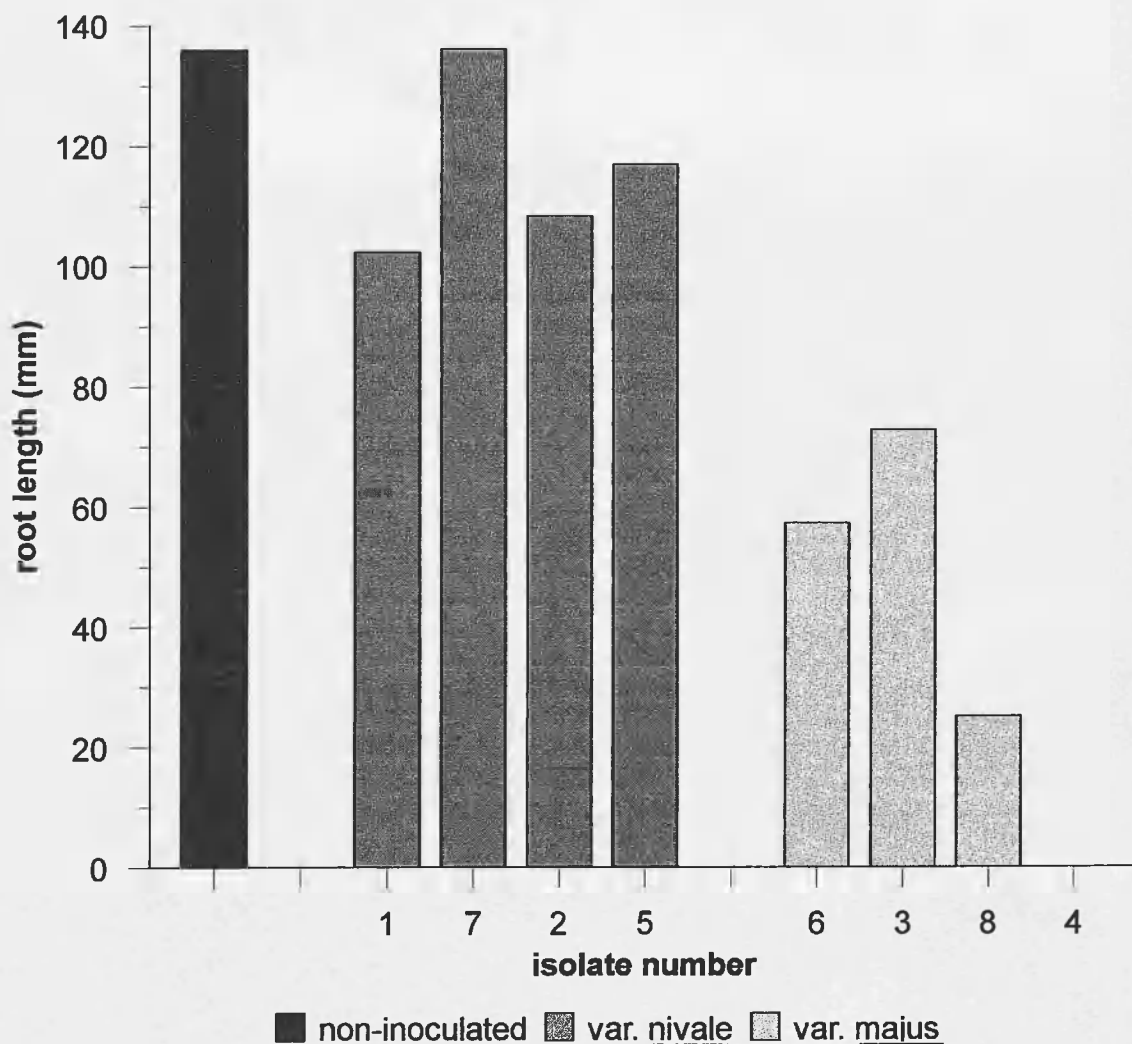


Figure 28 The root length of wheat seedlings following inoculation of seed (lot No. 4, see Table 3) with conidia from isolates of *Microdochium nivale* var. *majus* and *M. nivale* var. *nivale*. Seedlings were grown at 6°C. The SEM for non-inoculated seed = 3.27 and for inoculated seed = 4.62. The LSD ($P = 0.05$) for non-inoculated vs inoculated = 11.3 and for inoculated vs inoculated = 13.1; D of F = 61; CV = 14%.

Inoculation with isolates of *M. nivale* var. *majus* produced seedlings with significantly ($P < 0.001$) shorter coleoptiles, shoots and roots than the seedlings inoculated with isolates of *M. nivale* var. *nivale* or the non-inoculated controls (Figures 26, 27 and 28).

Variation between isolates with respect to their effect on coleoptile, shoot and root length was observed. Again, greater variation was observed between the seedlings inoculated with isolates *M. nivale* var. *majus* than with isolates of *M. nivale* var. *nivale*.

DISCUSSION

The relative occurrence of *M. nivale* var. *majus* and *M. nivale* var. *nivale* on naturally contaminated winter wheat seed is uncertain. Of the eight isolates of *M. nivale* recovered from U.K. wheat seed samples by Lees *et al.* (1995) all were classified as *M. nivale* var. *majus*. However, the present study has shown that both *M. nivale* var. *majus* and *M. nivale* var. *nivale* can be recovered from wheat seed. The majority of isolates were found to be *M. nivale* var. *majus* with only six of the 91 isolates classified being *M. nivale* var. *nivale*.

A possible reason for the predominance of *M. nivale* var. *majus* on wheat seed was proposed by Parry *et al.* (1995). They proposed that the increased perithecial production they observed *in vitro* from isolates of *M. nivale* var. *majus* may readily lead to ear infection via wind-borne ascospores. The more likely route for ear

infection by isolates of *M. nivale* var. *nivale* being via splash-borne conidia which is reliant on rain splash events for inoculum dispersion (Jenkinson and Parry, 1994). A similar situation occurs with respect to the infection of wheat ears by *F. graminearum*. Francis and Burgess (1977) reported the existence of two distinct populations of *F. graminearum* (Group 1 and Group 2). Group 2 isolates were considered homothallic perithecia producers and were commonly isolated from wheat ears, whereas Group 1 isolates were described as probably heterothallic or infertile and were commonly isolated from wheat stem-bases. However, like *M. nivale* var. *majus* and *M. nivale* var. *nivale*, both Group 1 and Group 2 have been associated with ear blight (Burgess *et al.*, 1987).

Differences between isolates of *M. nivale* with respect to perithecial production were also observed by Smith (1983). Smith observed that two *M. nivale* isolates recovered from winter wheat seeds from Norway readily produced perithecia *in vitro*. However, no perithecia were produced by isolates recovered from grasses, under similar conditions. Smith speculated that the isolates recovered from grasses were *M. nivale* var. *nivale* and those from wheat were *M. nivale* var. *majus*. However, Smith was unable to distinguish between the two groups of isolates on the basis of conidial morphology owing to an insufficient number of available cereal isolates. The results from the present study do add some weight to Smith's proposal that *M. nivale* var. *nivale* isolates are less likely to produce perithecia than *M. nivale* var. *majus* isolates and that turf grass isolates may more commonly be the former and cereal isolates more commonly the latter.

In addition, to being more common on wheat seeds, the results from this study also showed that isolates *M. nivale* var. *majus* were more pathogenic on wheat seedlings than isolates of *M. nivale* var. *nivale* under the test conditions applied. Differences in the pathogenicity of *M. nivale* isolates towards barley seedlings were observed by Perry and Al-Hashimi (1983) but no differentiation was made between var. *majus* and var. *nivale* isolates. Maurin (1993) worked with isolates classified as either *M. nivale* var. *majus* or *M. nivale* var. *nivale* and observed differences in pathogenicity between the two *M. nivale* varieties. He found that *M. nivale* var. *majus* showed only weak pathogenicity as compared to *M. nivale* var. *nivale* but did not state towards which plant this difference in pathogenicity was observed or how the test was performed. Subsequently Maurin *et al.* (1995) found no clear correlation between *M. nivale* variety and pathogenicity towards wheat seedlings (cv. Camp-Remy) inoculated with mycelial plugs at the two leaf stage. Therefore, although there is some evidence for the differential pathogenicity of the two *M. nivale* varieties on cereals it is not clear which *M. nivale* variety is more pathogenic on which cereal. In addition, the work in the present study was only performed with one wheat cultivar, Brigadier inoculated with macroconidia. It is possible that the pathogenicity of *M. nivale* isolates is different not only with respect to the cereal tested and the cultivar, but also the method and timing of inoculation.

The fact that *M. nivale* var. *majus* appears to be more common on naturally contaminated wheat seeds and more pathogenic towards wheat seedlings than *M. nivale* var. *nivale* may be important with respect to the control of seedling blight

symptoms in the field. Little is known about the relative effects of fungicides on the two varieties. Parry *et al.* (1995) observed that only two of the 237 isolates of *M. nivale* they tested were sensitive to benomyl, a chemical belonging to the MBC group of fungicides which can be found in cereal seed treatments. Such a fungicide would control isolates of *M. nivale* var. *nivale* sensitive to MBC's but not resistant isolates or isolates of the more frequently isolated *M. nivale* var. *majus*. The relative effects of other commonly used fungicides towards the two *M. nivale* varieties is unknown.

CHAPTER 8

**Chemical control of *Fusarium* seedling blight caused by natural *M.*
nivale contamination of wheat seeds**

INTRODUCTION

The chemical control of *Fusarium* seedling blight using fungicide seed treatments can significantly decrease the severity of visible symptoms (Chapter 1).

As discussed in previous chapters, environmental factors such as temperature and soil water (Chapter 5) and the percentage of seeds contaminated by *M. nivale* (Chapter 6) can have a marked effect on the severity of seedling blight symptoms. It is likely that disease severity will be greatest when a seed lot with a large percentage of seeds contaminated by *M. nivale* is sown into cold dry conditions where the rate of seedling emergence will be slow.

There is little information about the relationship between the efficacy of fungicide seed treatments and conditions conducive to seedling blight, for example increasing percentage *M. nivale* seed contamination and decreasing temperature.

The aims of the work in this chapter were to (i) investigate the relationship between the percentage of seeds naturally contaminated by *M. nivale* in a seed lot and the efficacy of a range of fungicide seed treatments, (ii) study the effect of temperature on fungicide seed treatment efficacy and, (iii) evaluate the effects of seed treatments in the field and to monitor *M. nivale* and *F. culmorum* stem-base infections throughout the growing season in established wheat plants.

MATERIALS AND METHODS

Details of the fungicides used as seed treatments in the following experiments are given in Table 19.

Table 19 Details of fungicides used as seed treatments

active ingredient(s)	formulation	concentration (g/l)	use rate (ml/100kg seed)
carboxin + thiabendazole	FS	360 + 20	250
flutriafol	FS	25	200
guazatine	LS	300	200
prochloraz	LS	200	200
fenpiclonil	FS	50	400
thiabendazole	FS	20	250
flutriafol + maneb	LS	25 + 400	200

(i) The Relationship between the Percentage of Naturally Contaminated Seeds (Inoculum Load) and the Efficacy of Fungicide Seed Treatments

Seed lots used

Winter wheat seed cv. Riband (seed lot Nos 20, 19, 17, 15 and 22 (Table 3))

was used in this study. Seed lots Nos 20, 19, 17, and 15 had been tested for *M. nivale* contamination previously and had 8%, 19%, 44% and 72% of seeds contaminated by *M. nivale* respectively (Chapter 3). Seed lot 22 was tested similarly (Chapter 2) and found to have no seeds contaminated by *M. nivale*.

Seed treatments used

Seed treatments containing carboxin + thiabendazole (TBZ), flutriafol, guazatine, prochloraz and fenpiclonil were used in this study. The seed treatments were applied to seed of each of the lots tested using a bench top Mini Rotostat (Marline (General Engineers) Limited, Watton, Norfolk, U.K.) at the rates given in Table 19. A sample of seed from each of the seed lots tested was left untreated to provide controls. A treatment list for the experiment is given in Table 20.

Sowing of seed

Following treatment with fungicide, seeds were sown at the rate of 10 seeds per jar in glass jars containing 60 g of sterilized soil-based compost (John Innes Seed) to a depth of 2 cm (Table 4). Three replicate jars were used for each treatment. The jars were then placed in an incubator set at 6°C according to a fully randomised design, and illuminated for 12 hours per day by Philips cool-white fluorescent tubes.

Table 20 Treatment numbers for treatments used to investigate the relationship between inoculum load and the efficacy of fungicide seed treatments for the control of *Microdochium nivale* seedling blight of winter wheat

Fungicide seed treatment	Percentage of seeds contaminated by <i>Microdochium nivale</i>				
	0	8	19	44	72
No fungicide	1	7	13	19	25
flutriafol	2	8	14	20	26
carboxin +TBZ	3	9	15	21	27
guazatine	4	10	16	22	28
prochloraz	5	11	17	23	29
fenpiclonil	6	12	18	24	30

Assessments of seedling emergence and seedling growth

The total number of emerged seedlings was counted daily. Seedling emergence was taken to be complete when no further seedlings emerged on five successive days. The mean seedling emergence time in days was calculated using Equation 1 (Chapter 2) (Khah *et al.*, 1986). The mean rate of emergence was calculated as the reciprocal of the mean seedling emergence time in days .

Following complete emergence, the length of each seedling's coleoptile, shoot and longest root was measured. The severity of disease symptoms on emerged seedlings was then assessed and a disease score was calculated (Chapter 2). Seedling dry weight was then measured following oven drying at 102°C to constant weight.

Data analysis

Seedling emergence, rate of seedling emergence, coleoptile length, shoot length, root length and dry weight data were analysed statistically using factorial ANOVA. *Microdochium nivale* contamination and fungicide seed treatment were treated as factors. Statistical analyses were performed on a personal computer using the program Statgraphics. Disease severity data were not analysed statistically using ANOVA because of the lack of variability in the data owing to few seedlings having visible disease symptoms. Mean disease severity values for each treatment are presented.

(ii) The Effect of Temperature on the Efficacy of Fungicide Seed Treatments

Seed lots and fungicide seed treatments used

Winter wheat seed cv. Riband (seed lot Nos 15 and 22 (Table 3) was used in this study. The two seed lots had been tested for *M. nivale* contamination previously and had 72% and 0% of seeds contaminated by *M. nivale* respectively (Chapter 6). Seed treatments containing flutriafol, guazatine, prochloraz and thiabendazole (TBZ), were applied to seed of each of the two seed lots with a bench top Mini Rotostat at the rates given in Table 19. A sample of seed from each of the seed lots used was left untreated to provide controls. A treatment list for the experiment is given in Table 21.

Seeds were sown at the rate of 10 seeds in glass jars containing 60 g of sterilized soil-based compost (John Innes Seed) to a depth of 2 cm (Table 4). Four

replicate jars were used for each seed lot/fungicide treatment. The jars were then placed in an incubator set at either 6°C, 8°C, 10°C or 12°C according to a fully randomised design, and illuminated for 12 hours per day by Philips cool-white fluorescent tubes.

Assessments of seedling emergence and seedling growth

The total number of emerged seedlings was counted daily. Seedling emergence was taken to be complete when no further seedlings emerged on five successive days. The mean seedling emergence time in days was calculated using Equation 1 (Chapter 2) (Khah *et al.*, 1986). The mean rate of emergence was calculated as the reciprocal of the mean seedling emergence time in days .

Following complete emergence, the length of each seedling's coleoptile was measured. Seedling stem-base infection was then assessed by excising stem-bases from seedlings, surface sterilizing them and then placing them onto PDA in Petri dishes prior to incubation at 15 °C. After 10-14 days the resultant fungal colonies were examined and identified (Chapter 2).

Data analysis

Seedling emergence, rate of seedling emergence, coleoptile length, coleoptile infection and disease severity data were analysed statistically using factorial ANOVA to determine the effects of *M. nivale* contamination, fungicide seed treatment and temperature on seedling blight disease severity and subsequent seedling growth.

Statistical analyses were performed on a personal computer using the software program Statgraphics. Data from treatments 21-40 (0% *M. nivale*) were omitted from the analyses of seedling emergence, coleoptile infection and disease severity because of the lack of variability in the data owing to no disease in these treatments. No chemical phytotoxicity or evidence of disease was seen in the omitted treatments.

Table 21 Treatment numbers for treatments used to investigate the relationship between temperature and the efficacy of fungicide seed treatments for the control of *Microdochium nivale* seedling blight of winter wheat

Seed treatment	Temperature (°C)			
	6	8	10	12
<i>72% M. nivale</i>				
Untreated	1	2	3	4
flutriafol	5	6	7	8
guazatine	9	10	11	12
prochloraz	13	14	15	16
thiabendazole	17	18	19	20
<i>0% M. nivale</i>				
Untreated	21	22	23	24
flutriafol	25	26	27	28
guazatine	29	30	31	32
prochloraz	33	34	35	36
thiabendazole	37	38	39	40

(iii) The Efficacy of Fungicide Seed Treatments in the Field

Seed lots and fungicide seed treatments used

Two seed lots of winter wheat cv. Riband and Lynx (Lot Nos 15 and 13, Table 3) were used in these field trials. The two seed lots had previously been tested for *M. nivale* contamination and had 72% and 38% of seeds contaminated by *M. nivale* respectively (Chapter 3). Seed treatments were applied to seed from each lot using a modified Mini Rotostat seed treater. Seed was treated with five seed treatments containing the fungicides; flutriafol + maneb, guazatine, prochloraz, fenpiclonil and carboxin + TBZ at the recommended rates (Table 19). Seed from each lot was left untreated to provide controls. A treatment list is given in Table 22.

Table 22 Treatment number and fungicide seed treatment used in field trials

Treatment No.	Fungicide seed treatment
1	Untreated
2	prochloraz
3	carboxin + TBZ
4	fenpiclonil
5	guazatine
6	flutriafol + maneb

Trial site details and sowing of seed

Two small plot field trials were sown in December 1993, trial No. 1 in Cambridgeshire and trial No. 2 in Lincolnshire. Site details are given in Table 23. Both trials were of a randomised block design with four blocks, and formed part of two Zeneca Crop Protection seed treatment efficacy trials. The trials were drilled into a wet and cold seedbed with a Hege small plot drill producing plots 1.5 m wide and 12 m long.

Table 23 Site details for the two seed treatment efficacy field trials

	Trial Number	
	1	2
Location	Little Abingdon, Camb.	Leadenham, Linc.
Crop	winter wheat	winter wheat
Cultivar	Lynx	Riband
Soil texture	Sandy clay loam	Sandy loam
Sowing date	07/12/93	01/12/93
Sowing rate	328 seeds/m ²	398 seeds/m ²

Assessments performed on field trials

A range of assessments were performed throughout the growth of the crop and are summarised in Table 24.

Crop establishment was assessed when the crop was at GS 11-12. The number

of plants on both sides of a 0.5 m stick, placed between two drill rows, was counted at four random points across the plot. The mean of the four values gave the number of plants per metre row in each plot.

The presence of *M. nivale* lesions on the stem-base was assessed at GS 11-12 and GS 30-31. Ten plants per plot were selected at random and the presence or absence of lesions noted.

The number of tillers per plant was assessed at GS 30-31. Ten plants per plot were selected at random and the number of tillers on each plant was recorded.

The number of ears per m² was assessed at GS 90 (trial No. 1) and GS 92 (trial No. 2). The number of ears in a 0.25 m² quadrat was counted at four points at random across the plot. These four values were added together to give the number of ears per m² in each plot.

Crop grain yield was assessed by harvesting whole plots using a Sampo 2010 small plot combine. Grain moisture content was also recorded using a Sinar Agritech moisture computer. Grain yield per plot was adjusted to t/ha at 85 % dry matter.

Stem-base infection by *M. nivale* and *F. culmorum* was assessed at GS 11-12, GS 30-31, GS 45 and GS 75, at both of the sites. Ten plants were selected at random from across the plot and following washing and surface sterilization the stem-bases were placed onto PDA in Petri dishes and incubated at 15°C for 10 - 14 days (Chapter 2). The resultant fungal colonies were then identified according to their colony and spore morphology.

Table 24 Assessment details for field trials No. 1 and 2

	Date	Assessment
<u>Trial No. 1 (Camb.)</u>	03/03/94	Crop establishment*
	24/03/94	Stem-base isolations
	04/05/94	Number of tillers/plant Stem-base isolations
	14/06/94	Stem-base isolations
	20/07/94	Crop ear count Stem-base isolations
	08/08/94	Grain yield and moisture content*
<u>Trial No. 2 (Linc.)</u>	10/03/94	Crop establishment*
	24/03/94	Stem-base lesions Stem-base isolations
	19/05/94	Number of tillers/plant Stem-base lesions Stem-base isolations
	14/06/94	Stem-base isolations
	20/07/94	Stem-base isolations
	30/08/94	Crop ear count* Grain yield and moisture content*

* Assessments performed by Zeneca Crop Protection experimentalists

Data analysis

Data were analysed using ANOVA and where the effect of treatment was found to be statistically significant ($P < 0.05$) LSD ($P = 0.05$) was used to differentiate between treatment means. Certain stem-base infection data were not analysed using ANOVA because of their lack of suitability for this analysis owing to few infections being detected. In such cases treatment means are presented.

RESULTS

(i) The Relationship between the Percentage of Naturally Contaminated Seeds (Inoculum Load) and the Efficacy of Fungicide Seed Treatments

Seedling emergence

As can be seen from Figure 29a, seed treatments containing carboxin + TBZ, guazatine, prochloraz and fenpiclonil all gave good control of pre-emergence seedling death. Seed not treated with fungicide and that treated with flutriafol produced fewer seedlings than the previous treatments. Most seedlings emerged (15.3 and 15.6 seedlings) from the two seed lots with little or no *M. nivale* contamination (0% and 8% respectively). Progressively fewer seedlings emerged as the percentage contamination of the seed lot increased from 19% (14.6 seedlings) to 72% (12.4 seedlings) (Figure 29b), especially for seed without fungicide and that treated with

flutriafol. This effect was highlighted by the statistically significant interaction observed between *M. nivale* contamination and seed treatment and can be seen in Figure 29c.

The rate of seedling emergence

The rate of seedling emergence following treatment with carboxin + TBZ and flutriafol was not significantly different ($P = 0.05$) from that of untreated seed (0.0368 day^{-1}). However, treatment with prochloraz reduced the rate of seedling emergence (0.0358 day^{-1}) and treatment with guazatine or fenpiclonil increased the rate of seedling emergence (0.0376 and 0.0380 day^{-1} respectively) with respect to the untreated ($P < 0.05$) (Figure 30a). Seedlings emerged fastest from seed with 0% *M. nivale* contamination (0.0375 day^{-1}) and slowest from seed with 72% *M. nivale* (0.0364 day^{-1}) (Figure 30b). The rate of seedling emergence decreased as the percentage of *M. nivale* contaminated seeds increased with the exception of seed lot No. 20 with 8% *M. nivale*.

Seedling coleoptile length

Seed treatment with an azole fungicide resulted in seedlings with short coleoptiles, treatment with prochloraz (23.6 mm) being significantly shorter than with flutriafol (30.0 mm). The other fungicides tested (fenpiclonil, carboxin + TBZ and guazatine) produced seedlings with coleoptiles significantly longer (34.4, 34.9 and 35.7 mm respectively) than the untreated (32.1 mm) (Figure 31a). Seed without

Figure 29a

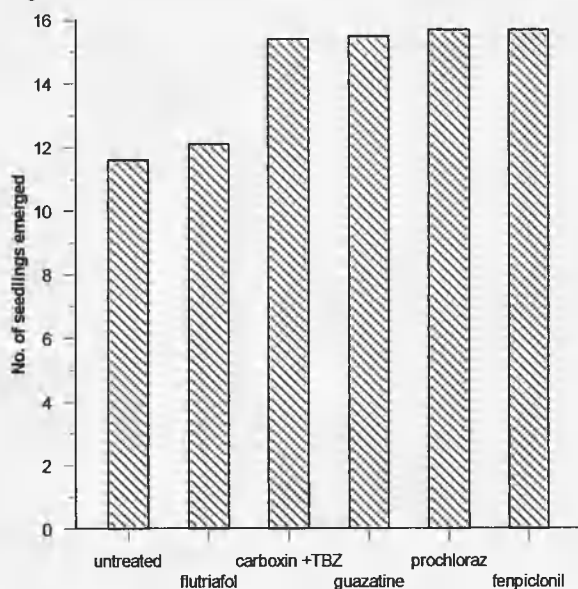


Figure 29b

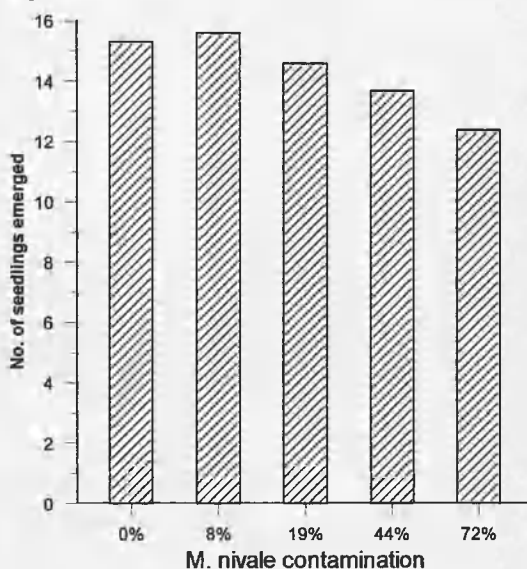


Figure 29c

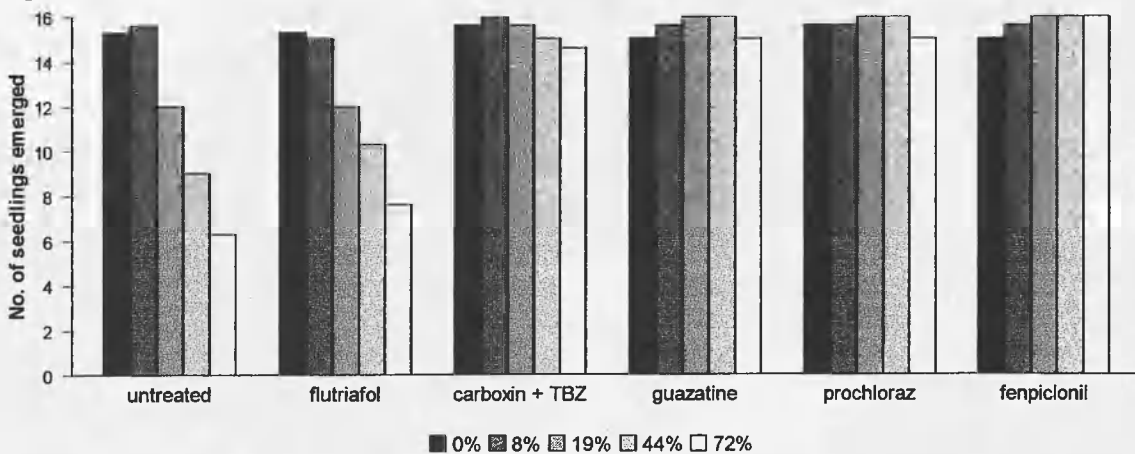


Figure 29 The effect of (a) fungicide seed treatment (SEM = 0.190), (b) natural *Microdochium nivale* seed contamination (SEM = 0.174) and (c) the relationship between fungicide seed treatment and *M. nivale* seed contamination (SEM = 0.43, LSD ($P = 0.05$) = 1.2) on the mean number of emerged wheat seedlings cv Riband (lot Nos 20, 19, 17, 15 and 22, see Table 3). D of F = 60; CV = 5.14%.

Figure 30a

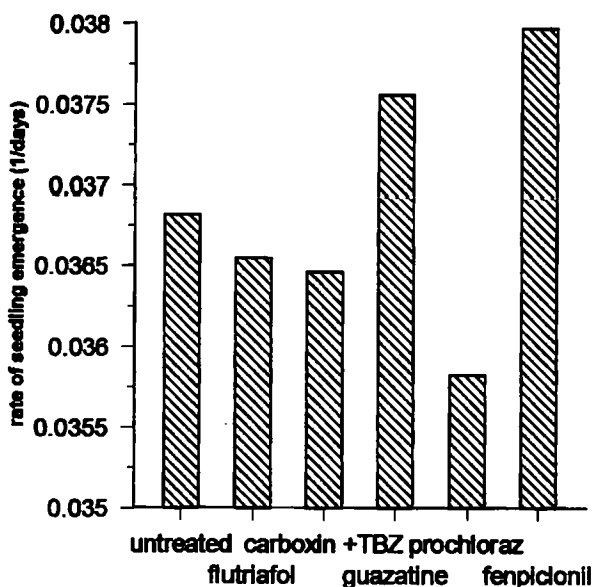


Figure 30b

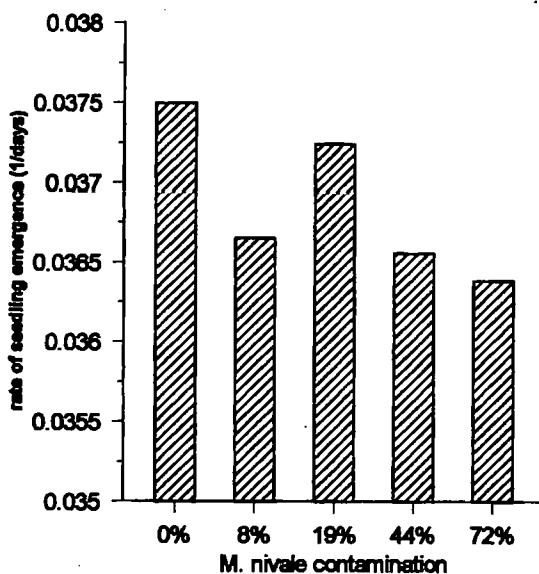


Figure 30 The effect of (a) fungicide seed treatment ($SEM = 2.5 \times 10^{-4}$; $LSD (P = 0.05) = 6.8 \times 10^{-4}$) and (b) natural *Microdochium nivale* seed contamination ($SEM = 2.2 \times 10^{-4}$; $LSD (P = 0.05) = 6.2 \times 10^{-4}$) on the rate of emergence of wheat seedlings cv Riband (lot Nos 20, 19, 17, 15 and 22, see Table 3). D of F = 60; CV = 2.6%.

M. nivale contamination produced seedlings with longer coleoptiles (35.4 mm) than seed contaminated by *M. nivale* (30.1 to 31.9 mm) (Figure 31b). A significant interaction was observed between *M. nivale* contamination and seed treatment and this can be seen in Figure 31c.

Seedling shoot length

Seed treated with flutriafol and that not treated with a fungicide produced seedlings with the shortest shoots (85.5 and 87.5 mm respectively). Treatment with fenpiclonil resulted in seedlings with significantly longer shoots (111.2 mm) than those of the other treatments (Figure 32a). Seed without *M. nivale* contamination produced seedlings with longer shoots (105.3 mm) than with *M. nivale* contamination (Figure 32b). Seed with 72% *M. nivale* contamination produced seedlings with the shortest shoots (85.9 mm). A significant interaction was observed between *M. nivale* contamination and seed treatment and this can be seen in Figure 32c. Untreated seed produced seedlings of shorter shoot length as the percentage of *M. nivale* contaminated seed in each lot increased, whereas the effect of *M. nivale* contamination on shoot length was eliminated with fenpiclonil treatment.

Seedling root length

The two azole treatments, flutriafol and prochloraz, produced seedlings with roots which were not significantly different in length ($P = 0.05$) to those from untreated seed. However, treatment with guazatine, carboxin + TBZ and fenpiclonil

Figure 31a

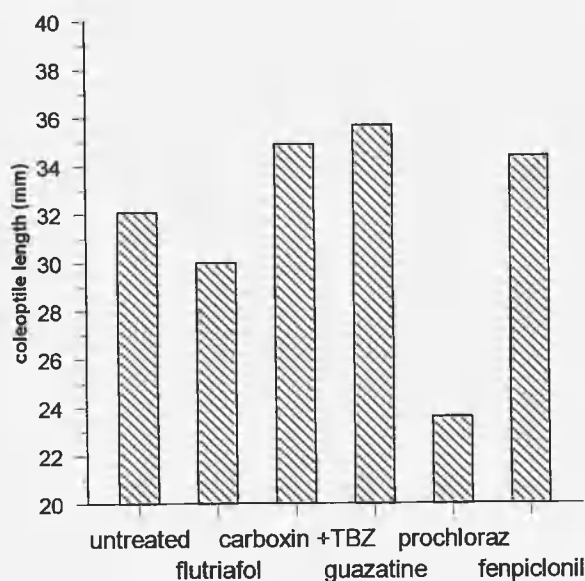


Figure 31b

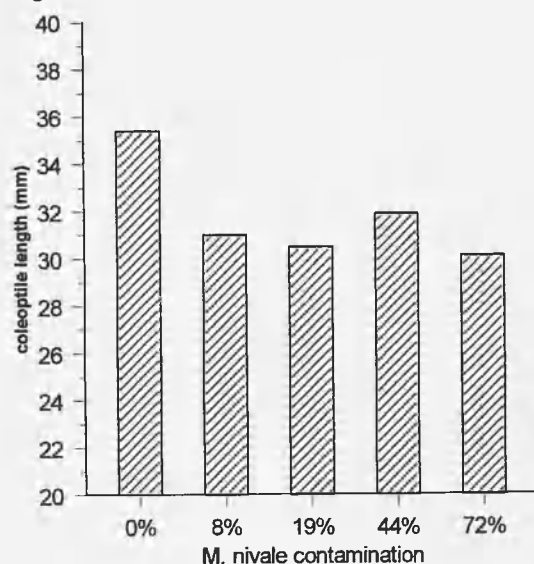


Figure 31c

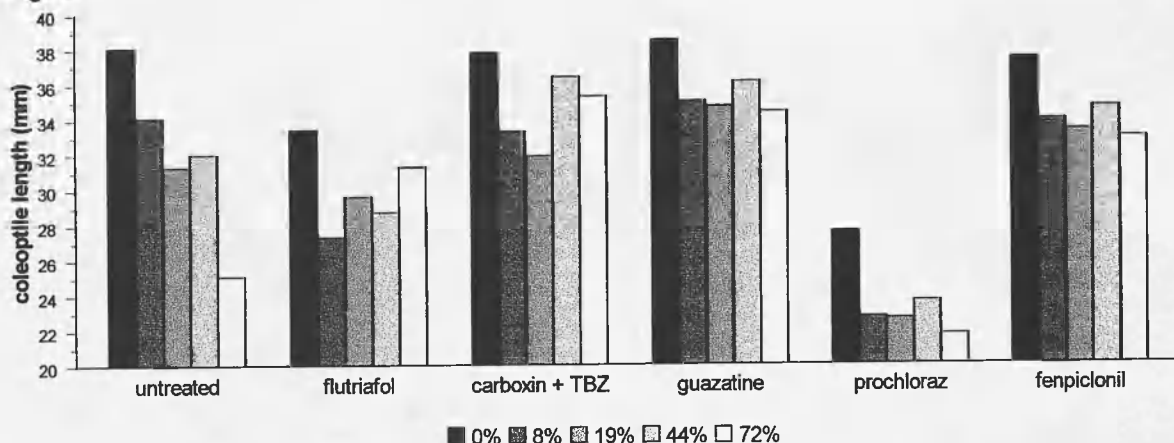


Figure 31 The effect of (a) fungicide seed treatment (SEM = 0.45), (b) natural *Microdochium nivale* seed contamination (SEM = 0.41) and (c) the relationship between fungicide seed treatment and natural *M. nivale* seed contamination (SEM = 1.0; LSD ($P = 0.05$) = 2.8) on the coleoptile length (mm) of emerged wheat seedlings cv Riband (lot Nos 20, 19, 17, 15 and 22, see Table 3). D of F = 60; CV = 5.5%.

Figure 32a

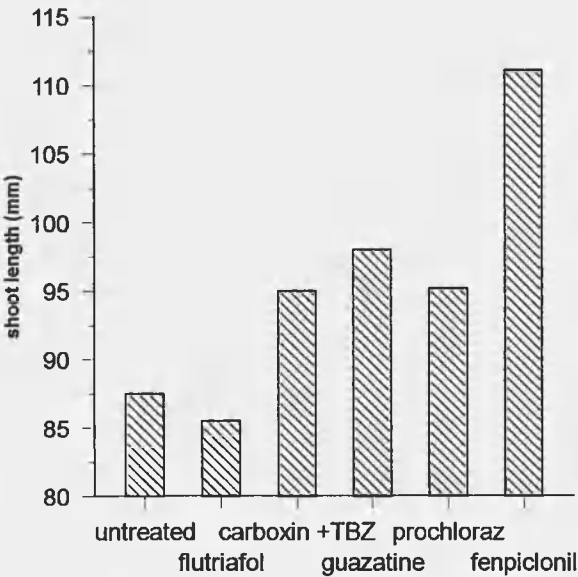


Figure 32b

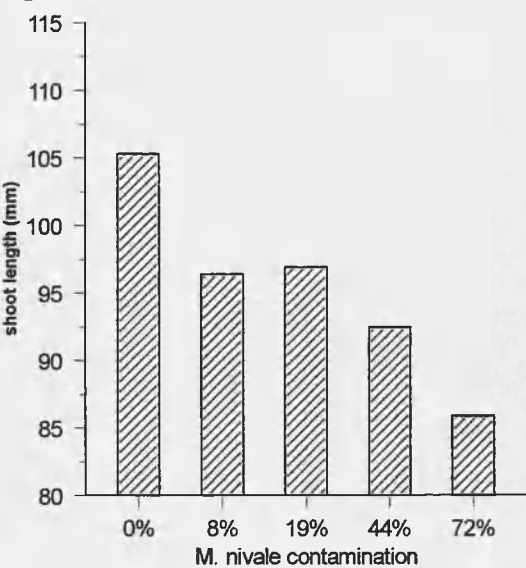


Figure 32c

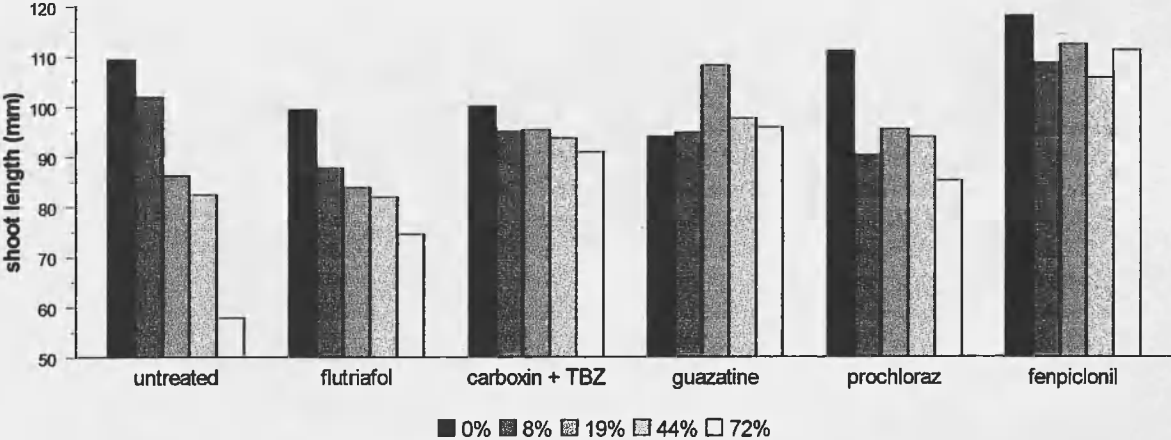


Figure 32 The effect of (a) fungicide seed treatment (SEM = 2.32), (b) natural *Microdochium nivale* seed contamination (SEM = 2.12) and (c) the relationship between fungicide seed treatment and natural *M. nivale* seed contamination (SEM = 5.19; LSD ($P = 0.05$) = 14.4) on the shoot length (mm) of emerged wheat seedlings cv Riband (lot Nos 20, 19, 17, 15 and 22, see Table 3). D of F = 60; CV = 9.4%.

produced seedlings with significantly longer roots ($P = 0.05$) (Figure 33a). Seed with 72% *M. nivale* contaminated seed produced seedlings with significantly shorter ($P = 0.05$) roots than seedlings from the other seed lots tested (Figure 33b).

Seedling dry weight

Seed not treated with fungicide and that treated with flutriafol produced seedlings with a significantly lower dry weight (0.40 g and 0.39 g respectively) than the other fungicide treatments tested ($P = 0.05$). Treatment with guazatine (0.55 g) and carboxin + TBZ (0.57 g) produced seedlings with the greatest dry weight (Figure 34a). As can be seen in Figure 34b, seed with 8% of seeds contaminated by *M. nivale* produced seedlings with the greatest dry weight (0.58 g) and seed with 19% *M. nivale* produced seedlings with the least (0.42 g) ($P = 0.05$). A significant interaction was observed between *M. nivale* contamination and seed treatment. Seedling dry weight decreased with increasing *M. nivale* contamination when seed was either not treated with fungicide or was treated with flutriafol. The weight of seedlings produced following treatment with the other fungicides tested was unaffected by increasing *M. nivale* contamination (Figure 34c).

A summary of F ratios and statistical significance (P) for the analysed data are presented in Appendix 1.

Figure 33a

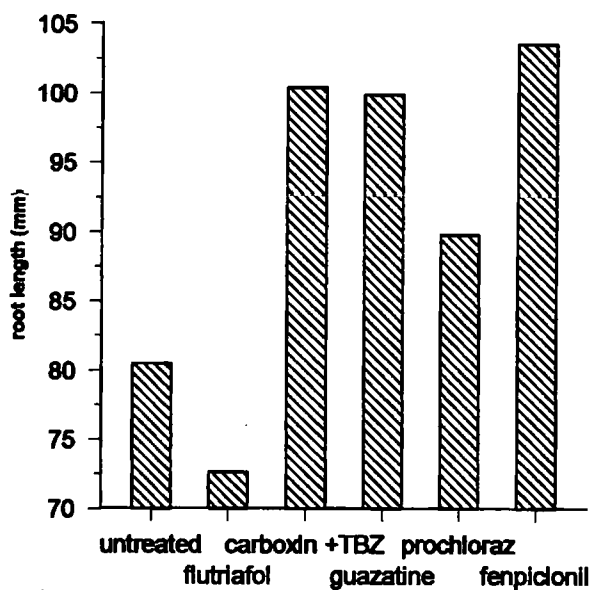


Figure 33b

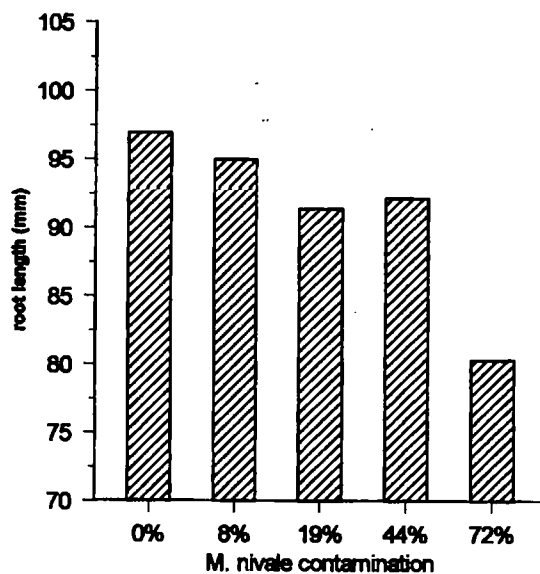


Figure 33 The effect of (a) fungicide seed treatment (SEM = 4.22; LSD ($P = 0.05$) = 11.7) and (b) natural *Microdochium nivale* seed contamination (SEM = 3.85; LSD ($P = 0.05$) = 10.7) on the root length (mm) of emerged wheat seedlings cv Riband (lot Nos 20, 19, 17, 15 and 22, see table 3). D of F = 60; CV = 17.9%.

Figure 34a

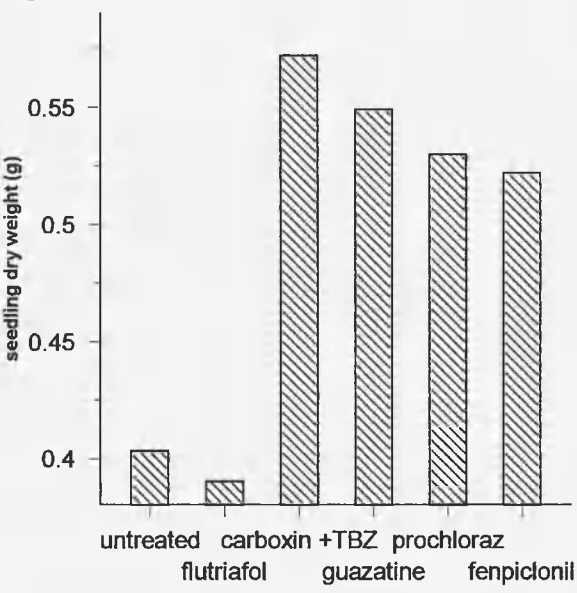


Figure 34b

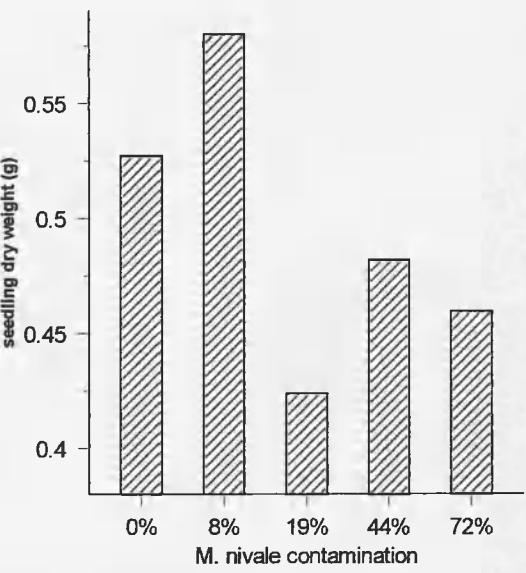


Figure 34c

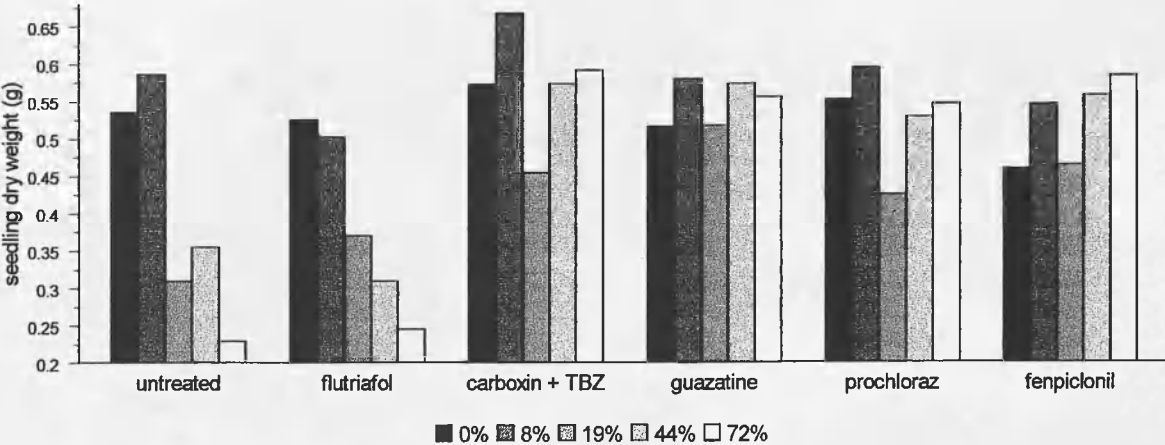


Figure 34 The effect of (a) fungicide seed treatment (SEM = 0.0114), (b) natural *Microdochium nivale* seed contamination (SEM = 0.0104) and (c) The relationship between fungicide seed treatment and natural *M. nivale* seed contamination (SEM = 0.0254, LSD ($P = 0.05$) = 0.07) on the dry weight of emerged wheat seedlings cv Riband (lot Nos 20, 19, 17, 15 and 22, see Table 3). D of F = 60; CV = 8.9%.

(ii) The Effect of Temperature on the Efficacy of Fungicide Seed Treatments

Seedling emergence

Seedling emergence from seed with no *M. nivale* contamination was good irrespective of temperature or fungicide seed treatment application. A minimum of 9 of the 10 seedlings sown emerged in each of the treatments' replicates.

However, significant differences ($P < 0.001$) were observed between temperature and fungicide treatments when *M. nivale* contaminated seed was sown. Overall, seedling emergence increased from 6.3 seedlings at 6°C to 9.0 seedlings at 12°C (Figure 35b). Seed not treated with fungicide and that treated with TBZ produced the fewest seedlings (6.5 and 6.6 seedlings respectively). Treatment with flutriafol resulted in significantly greater number of emerged seedlings (8.1 seedlings) with guazatine and prochloraz treatments producing the greatest number of emerged seedlings, 9.3 and 9.6 respectively (Figure 35a). A significant interaction was observed between temperature and fungicide treatment and this can be seen in Figure 35c. Seedling emergence decreased with decreasing temperature when seed was not treated with a fungicide and treated with TBZ or flutriafol, whereas no reduction in emergence was seen following treatment with guazatine or prochloraz.

Rate of seedling emergence

Treatment with prochloraz resulted in slower emergence than treatment with

Figure 35a

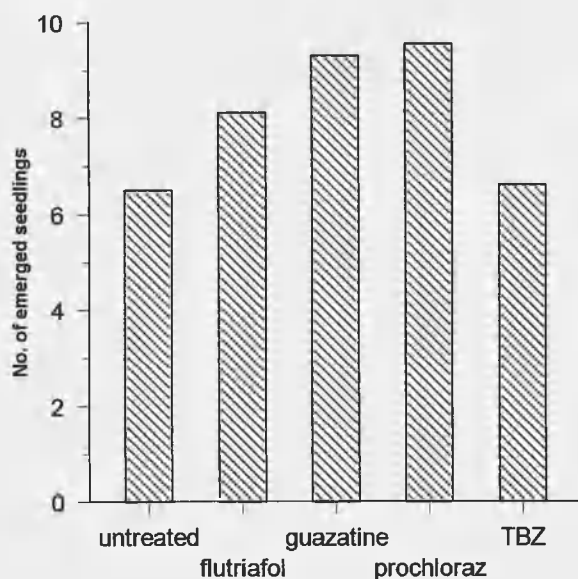


Figure 35b

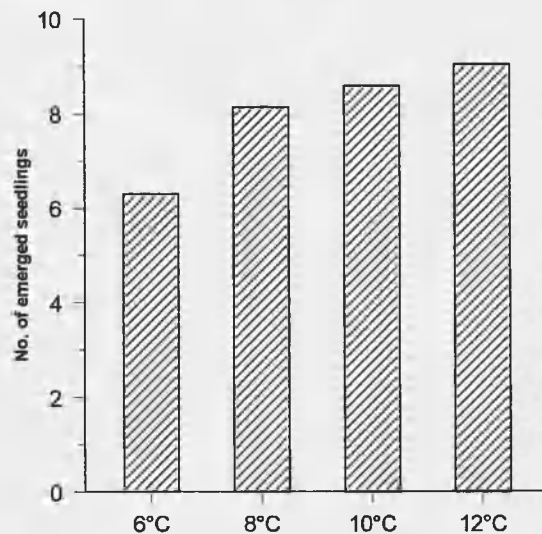


Figure 35c

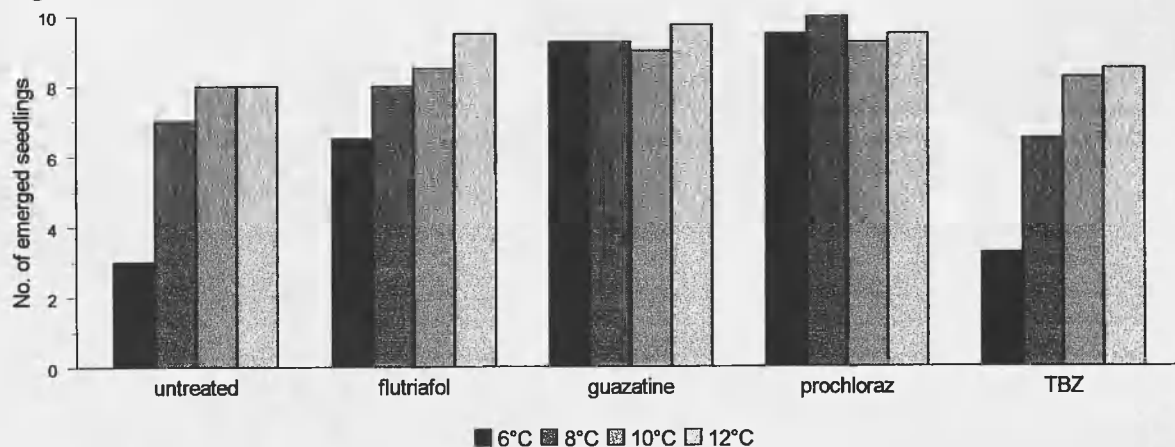


Figure 35 The effect of (a) fungicide seed treatment (SEM = 0.341), (b) temperature (SEM = 0.305) and (c) The relationship between fungicide seed treatment and temperature (SEM = 0.682; LSD ($P = 0.05$) = 1.36) on the emergence of wheat seedlings cv Riband (lot No. 15, see Table 3). D of F = 60; CV = 17%.

TBZ, flutriafol or no fungicide, whilst treatment with guazatine greatly increased the rate of seedling emergence (Figure 36a). As Figure 36b shows, overall, the rate of seedling emergence decreased from 0.03 days⁻¹ at 12°C to 0.06 days⁻¹ at 6°C. There was a significant interaction between *M. nivale* seed contamination and fungicide seed treatment which can be seen in Figure 36c. The rate of seedling emergence was slower with *M. nivale* contaminated than non-contaminated seed for all of the fungicide treatments with the exception of guazatine. Treatment with guazatine resulted in a much faster rate of emergence from the contaminated seed than the non-contaminated seed.

Seedling coleoptile length

Seed with no *M. nivale* contamination produced seedlings with longer coleoptiles (34.7 mm) than seed contaminated by *M. nivale* (27.0 mm). Treatment with fungicide had a marked effect on coleoptile length. Coleoptiles were shortest with the two azole treatments flutriafol (24.9 mm) and prochloraz (27.3 mm) and longest following treatment with guazatine (37.2 mm). There was little difference between the length of coleoptiles following treatment with TBZ or in the absence of fungicide (32.8 mm and 32.2 mm respectively) (Figure 37a). The effect of temperature on coleoptile length was less clear with the longest coleoptiles being produced at 6°C and 12°C, 33.6 mm and 34.2 mm respectively. Seedlings with the shortest coleoptiles occurred at 8°C (23.4 mm) (Figure 37b). Significant interactions were observed between each of the main factors; *M. nivale* seed contamination,

fungicide seed treatment and temperature (Appendix 2).

Seedling stem-base infection by *M. nivale*

No stem-base infection was observed in seedlings from the non-contaminated seed lot. However, temperature and fungicide seed treatment had a significant effect on the infection of seedling stem-bases by *M. nivale* from contaminated seed. Treatment with prochloraz and TBZ produced a similar number of infected seedlings as seed not treated with a fungicide (5.2, 4.7 and 4.7 respectively). Fewer infected seedlings (2.9) were observed following treatment with guazatine. Seed treated with flutriafol produced the greatest number of infected seedlings (7.5) (Figure 38a). Overall, the greatest number of infected seedlings (7.5) occurred at 8°C and the least (3.2) at 6°C. (Figure 38b). A significant interaction was observed between temperature and fungicide treatment which can be seen in Figure 38c. Following treatment with prochloraz and guazatine the greatest number of seedlings with infected stem-bases was observed at 8°C. This pattern of seedling infection with temperature was different for the other treatments where no obvious peak in seedling infection was observed at 8°C.

Figure 36a

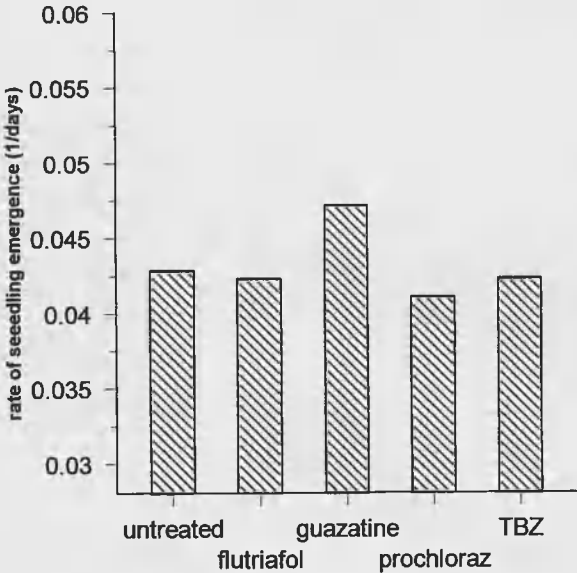


Figure 36b

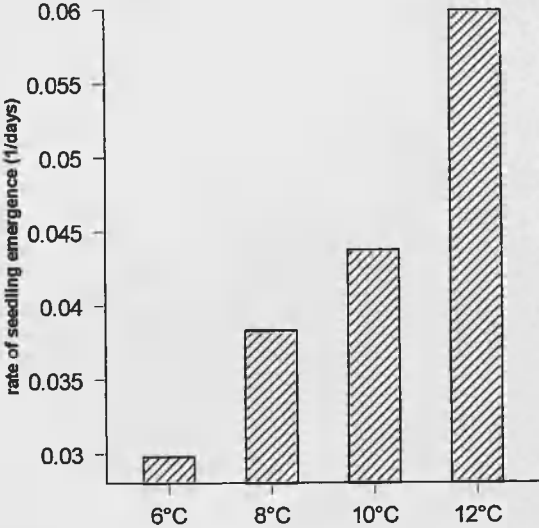


Figure 36c

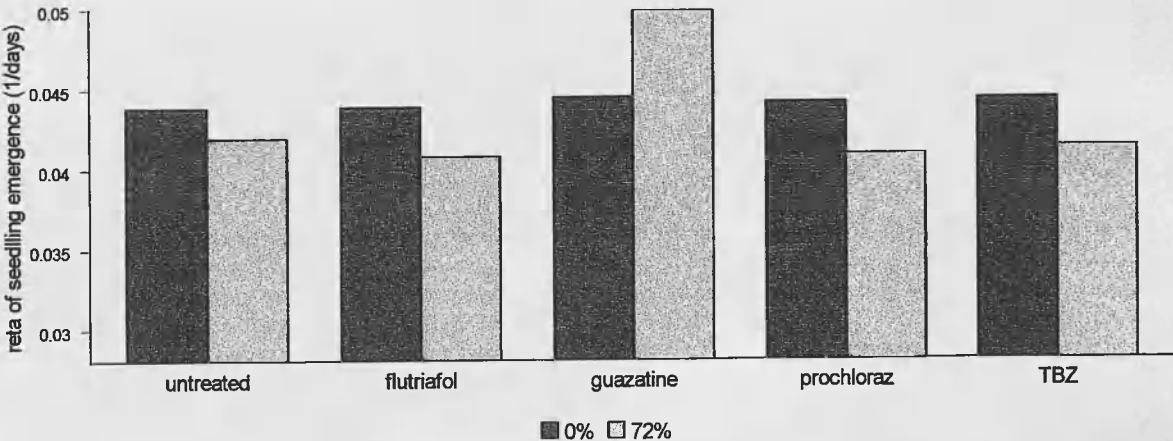


Figure 36 The effect of (a) fungicide seed treatment ($SEM = 8.7 \times 10^{-4}$), (b) temperature ($SEM = 7.79 \times 10^{-4}$) and (c) The relationship between fungicide seed treatment and natural *Microdochium nivale* seed contamination ($SEM = 1.23 \times 10^{-3}$; $LSD (P = 0.05) = 3.41 \times 10^{-3}$) on the emergence of wheat seedlings cv Riband (lot No. 15, see Table 3). D of F = 60; CV = 3.6%.

Figure 37a

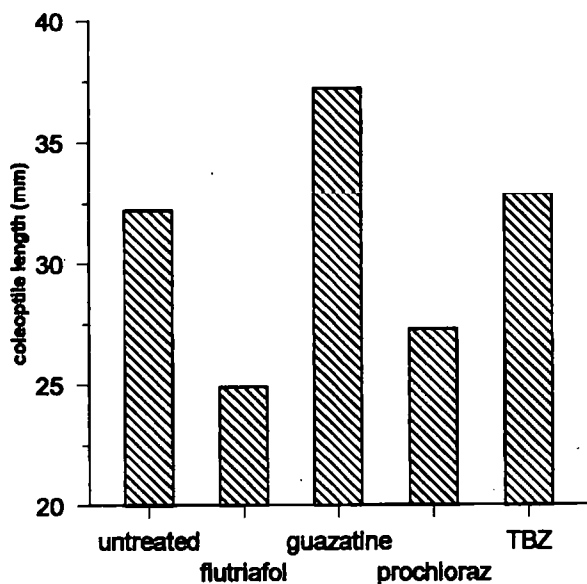


Figure 37b

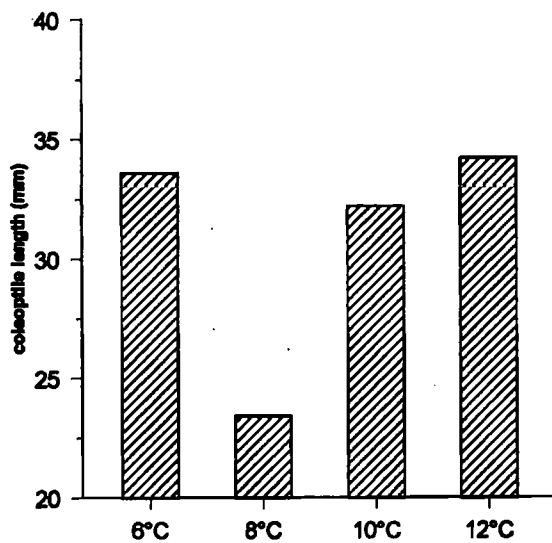


Figure 37 The effect of (a) fungicide seed treatment (SEM = 0.505) and (b) temperature (SEM = 0.452) on the coleoptile length of wheat seedlings cv. Riband (lot No. 15, see Table 3). D of F = 120; CV = 9.2%.

Figure 38a

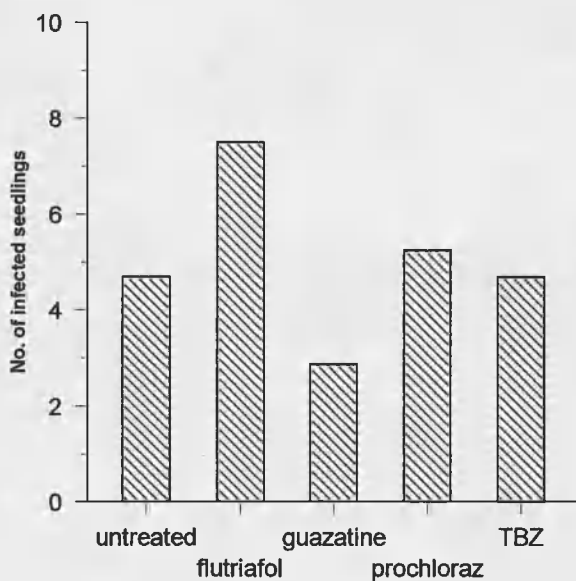


Figure 38b

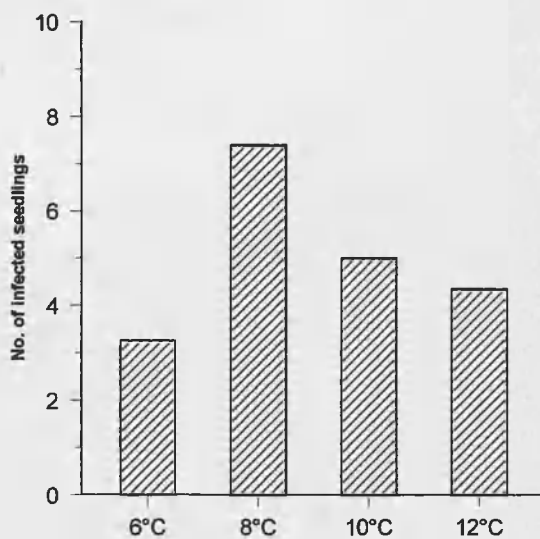


Figure 38c

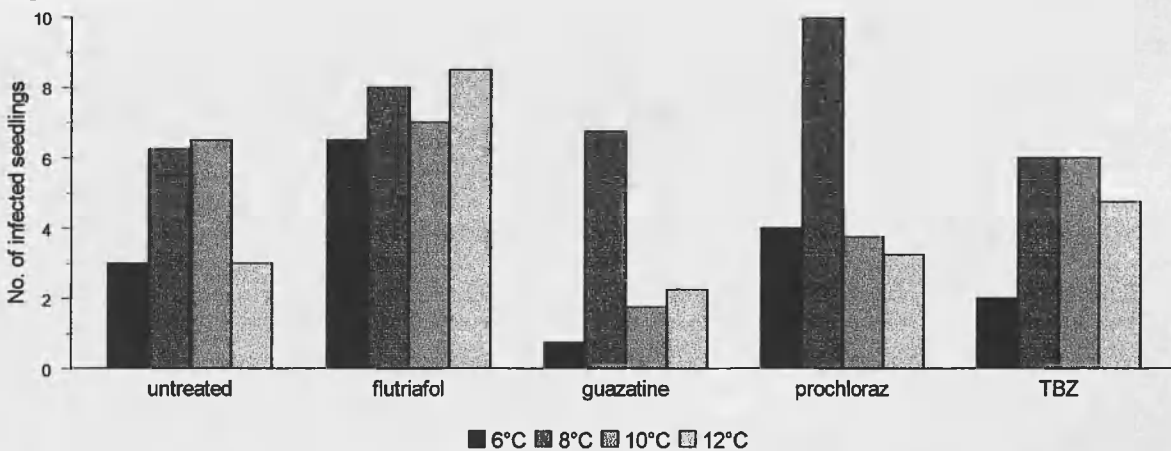


Figure 38 The effect of (a) fungicide seed treatment (SEM = 0.417), (b) temperature (SEM = 0.373) and (c) The relationship between fungicide seed treatment and natural *Microdochium nivale* seed contamination (SEM = 0.834, LSD ($P = 0.05$) = 2.31) on the infection of wheat seedling coleoptiles cv Riband (lot No. 15, see Table 3) D of F = 60; CV = 33%.

Seedling disease severity

Disease symptoms were not observed on seedlings from seeds with no *M. nivale* contamination. Disease symptoms were most severe on emerged seedlings from seed not treated with a fungicide (40.8), and that treated with flutriafol (38.5). The least severe disease symptoms were seen on seedlings following treatment with guazatine (13.8) and prochloraz (18.6) (Figure 39a). Overall, *M. nivale* disease symptoms on emerged seedlings were most severe (65.2) at 8°C. Disease symptoms were common at 6°C but not at 10°C or 12°C (Figure 39b).

A summary of F ratios and statistical significance (*P*) for the analysed data are presented in Appendix 2. *Microdochium nivale* contamination was not included as a factor in the analysis of seedling emergence, seedling stem-base infection or disease index data, and F ratios are not presented. *Microdochium nivale* contamination was omitted as a factor owing to the lack of seedling disease in the absence of the pathogen. Statistically significant differences ($P < 0.05$) were observed for fungicide seed treatment and temperature main effects in each analysis. *Microdochium nivale* contamination had a significant effect on seedling coleoptile length. Statistically significant interactions ($P < 0.05$) between main effects were also observed in all but the disease index data.

Figure 39a

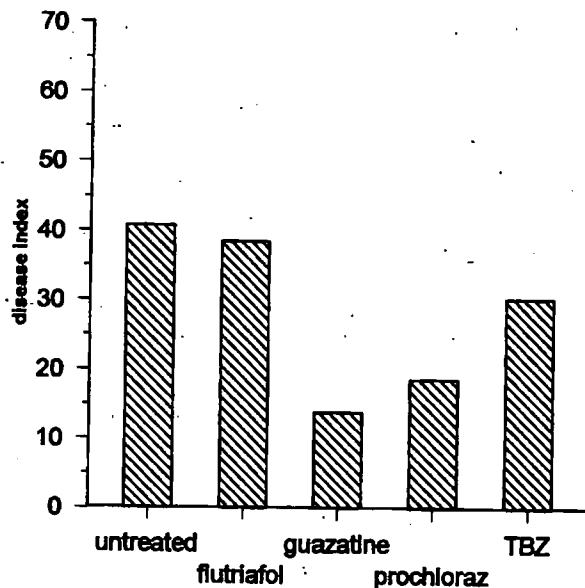


Figure 39b

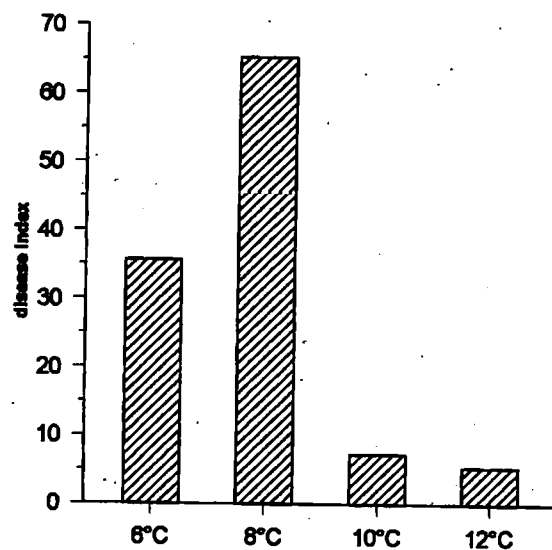


Figure 39 The effect of (a) fungicide seed treatment (SEM = 4.05) and (b) temperature (SEM = 3.62) on disease severity of emerged wheat seedlings cv Riband (lot No. 15, see Table 3). D of F = 60; CV = 57%.

(iii) The Efficacy of Fungicide Seed Treatments in the Field

Fungicide seed treatment had a significant effect ($P = 0.05$) on crop establishment, tiller number, ear number and yield at both trial sites with the exception of yield at site No. 1 where no significant difference ($P = 0.05$) was observed between fungicide treatment and yield. Significant differences between treatments with respect to *M. nivale* lesions on the stem-base at GS 10-11 and GS 30-31 were seen at trial site No. 2. No *M. nivale* lesions were seen at trial site No. 1 and no assessments were made.

Stem-base infection by *M. nivale* was significantly ($P = 0.05$) affected by fungicide seed treatment at GS 10-11 at trial site No. 1 and GS 10-11, 45 and 75 at trial site No. 2. Stem-base infection by *F. culmorum* was significantly affected by fungicide seed treatment at GS 30-31 at trial site No. 2. A summary of F ratios and statistical significance (P) for the analysed stem-base infection data are presented in Appendix 3.

Crop establishment

At site No. 1, treatment with fungicide increased the number of established seedlings with respect to those without a seed treatment. No significant differences were observed between fungicide treatments (Table 25). At site No. 2, seedling establishment in the untreated plots was much lower than at site No. 1 (2.5 and 15.1 plants per m row respectively), and again treatment with fungicide increased the

number of established seedlings with respect to those without a seed treatment (Table 26). Significant differences ($P = 0.05$) were also seen between fungicide treatments. Treatments containing flutriafol + maneb and carboxin + TBZ produced fewer seedlings than the other fungicide treatments.

Tiller number per plant

At site No. 1, treatment with fungicide decreased the number of tillers per plant with the exception of treatment with flutriafol + maneb which was not significantly different from the untreated. No significant difference in tiller number was seen between the other fungicide treatments (Table 25).

At site No. 2, treatment with fungicide, including flutriafol + maneb, reduced tiller number. There were significant differences between fungicide treatments. Treatment with guazatine and fenpiclonil resulted in plants with fewer tillers (4.6 and 4.8) than treatment with carboxin + TBZ 6.6) (Table 26).

Crop ear count

At site No. 1, treatment with fungicide increased the number of ears per square metre over that of the untreated with the exception of carboxin + TBZ. The only significant difference between fungicide treatments was that between carboxin + TBZ and guazatine. Treatment with guazatine resulted in 357 ears/m² and carboxin + TBZ 321 ears/m² (Table 25).

At site No. 2 treatment with fungicide increased the number of ears per square

metre over that of the untreated. Treatment with carboxin + TBZ again resulted in the lowest ear count from the fungicide treatments, but at this site it produced significantly fewer ears (189.2 ears/m²) than treatment with prochloraz (227.8 ears/m²) and fenpiclonil (234.2 ears/m²) (Table 26).

Grain yield and moisture content

Little difference was seen in grain moisture content between treatments at both sites. Moisture content ranged from 15.2% to 15.3% at site 1 and 15.8% to 15.9% at site 2. Significant differences between treatments for yield (t/ha @ 85% dry matter) were shown at site No. 2 with fungicide seed treatment increasing grain yield over that of the untreated (2.9 t/ha). However, there were no significant differences between fungicide treatments (Table 26). No significant differences were seen at site No. 1 (Table 25).

Plants with *M. nivale* lesions on the stem-base

Microdochium nivale lesions were only observed on the stem-base of plants at site No. 2 and significant ($P = 0.05$) treatment effects were seen. At GS 10-11 significantly more plants had lesions in flutriafol + maneb, carboxin + TBZ and untreated plots than the other fungicide treatments (Figure 40a). At GS 30-31 significantly more plants with lesions were seen in fenpiclonil (5.5) and flutriafol + maneb (6.0) treated plots than in untreated (3.0) plots. The fewest of plants with lesions occurred in the prochloraz treated plots (1.8) (Figure 40b).

Table 25 Plant count, tillers per plant, ear count and grain yield at trial site No. 1 (for details of site, see Table 24).

Fungicide seed treatment	Plant count (plants/m)	No. of tillers / plant (GS 30/31)	Ear count (ears/0.25m ²)	Yield @ 15% m.c. (t/ha)
Untreated	15.1	8.4	294.0	5.87
prochloraz	30.1	6.5	337.0	6.52
carboxin + TBZ	25.6	6.8	321.0	6.39
fenpiclonil	28.8	5.7	346.2	6.52
guazatine	29.2	6.3	357.0	6.48
flutriafol + maneb	30.0	8.9	346.2	6.48
LSD ($P = 0.05$)	4.6	1.2	34.3	N/A
SEM	2.54	0.42	11.38	0.167
D of F	15	15	15	15
CV	19	12	7	5

Table 26 Plant count, tillers per plant, ear count and grain yield at trial site No. 2 (for details of site, see Table 24).

Fungicide seed treatment	Plant count (plants/m)	No. of tillers \ plant (GS 30/31)	Ear count (ears/0.25m ²)	Yield @85% m.c. (t/ha)
Untreated	2.5	7.9	28.8	2.9
prochloraz	27.9	5.4	227.8	8.9
carboxin + TBZ	23.6	6.6	189.2	8.1
fenpiclonil	30.4	4.8	234.2	9.2
guazatine	30.4	4.6	201.8	8.7
flutriafol + maneb	21.8	5.8	203.2	8.2
LSD ($P = 0.05$)	5.9	1.3	33.3	1.1
SEM	1.96	0.43	11.00	0.37
D of F	15	15	15	15
CV	17	15	12	10

Figure 40a

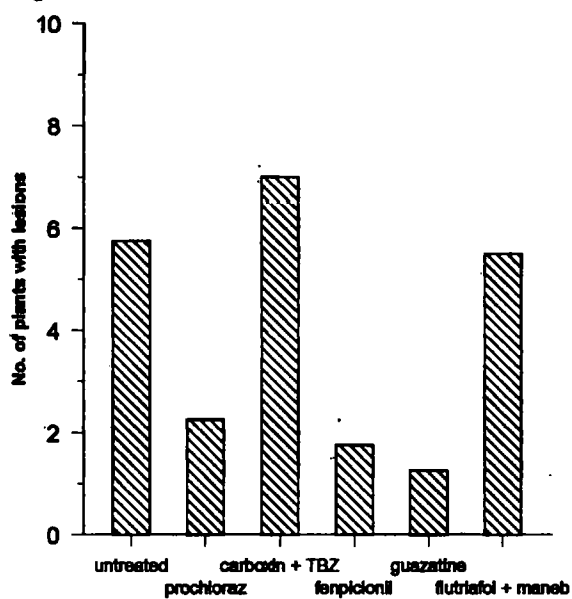


Figure 40b

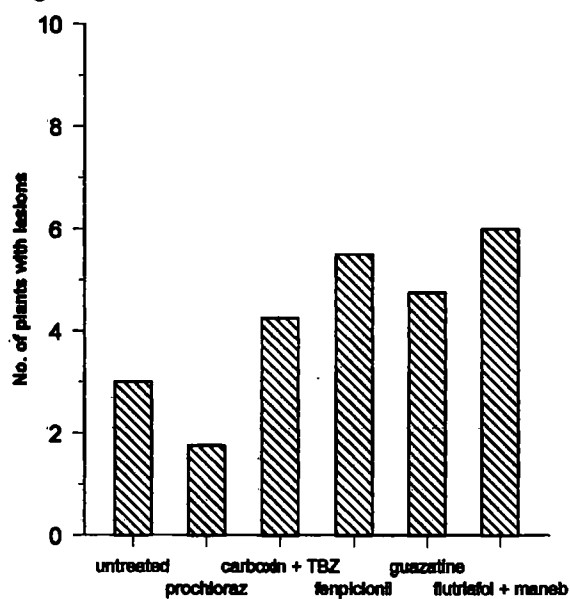


Figure 40 The number of wheat plants out of ten with *Microdochium nivale* lesions on the stem-base at trial site No. 2 (for details of site, see Table 24) at (a) GS 10-11 (SEM = 0.52; LSD ($P = 0.05$) = 1.6; D of F = 15; CV = 27%), (b) GS 30-31 (SEM = 0.59; LSD ($P = 0.05$) = 1.8; D of F = 15; CV = 28%).

Stem-base infection by *M. nivale* and *F. culmorum*

At site No. 1, the greatest number of plants with stem-base *M. nivale* infection occurred at GS 10-11. Flutriafol + maneb, carboxin + TBZ and untreated plots had significantly more ($P = 0.05$) stem-base infection (4.5, 5.8 and 7.0 plants respectively) than the other treatments (1.2 plants). Little stem-base infection was detected at GS 30-31, with none being detected in prochloraz, carboxin + TBZ and flutriafol + maneb treated plots. More stem-base infection was detected at GS 45 (1.8 to 3.8 plants). However, there was no significant difference ($P = 0.05$) between treatments. The number of plants with stem-base infection again declined and at GS 75 a maximum of 1.5 infected plants was observed in carboxin + TBZ plots (Figure 41).

At site No. 2, the greatest number of plants with stem-base *M. nivale* infection at GS 10-11 were again in flutriafol + maneb, carboxin + TBZ and untreated plots (2.0, 4.0 and 3.2 plants respectively). However, unlike site No. 1, *M. nivale* stem-base infection did not decrease with time. *Microdochium nivale* was detected in stem-bases at GS 30-31 in all plots although no significant difference between treatments was observed. Significant differences between treatments were observed at GS 45. Most stem-base infection was detected in fenpiclonil (3.0), guazatine (5.5) and carboxin + TBZ (6.0) treated plots, although only carboxin + TBZ had significantly more ($P = 0.05$) plants with stem-base infection than the other treatments. Stem-base infection was greatest at GS 75 with all but the prochloraz treated plots having at least 5 infected plants per plot (Figure 42).

At site No. 1, no *F. culmorum* stem-base infection was observed at GS 10-11.

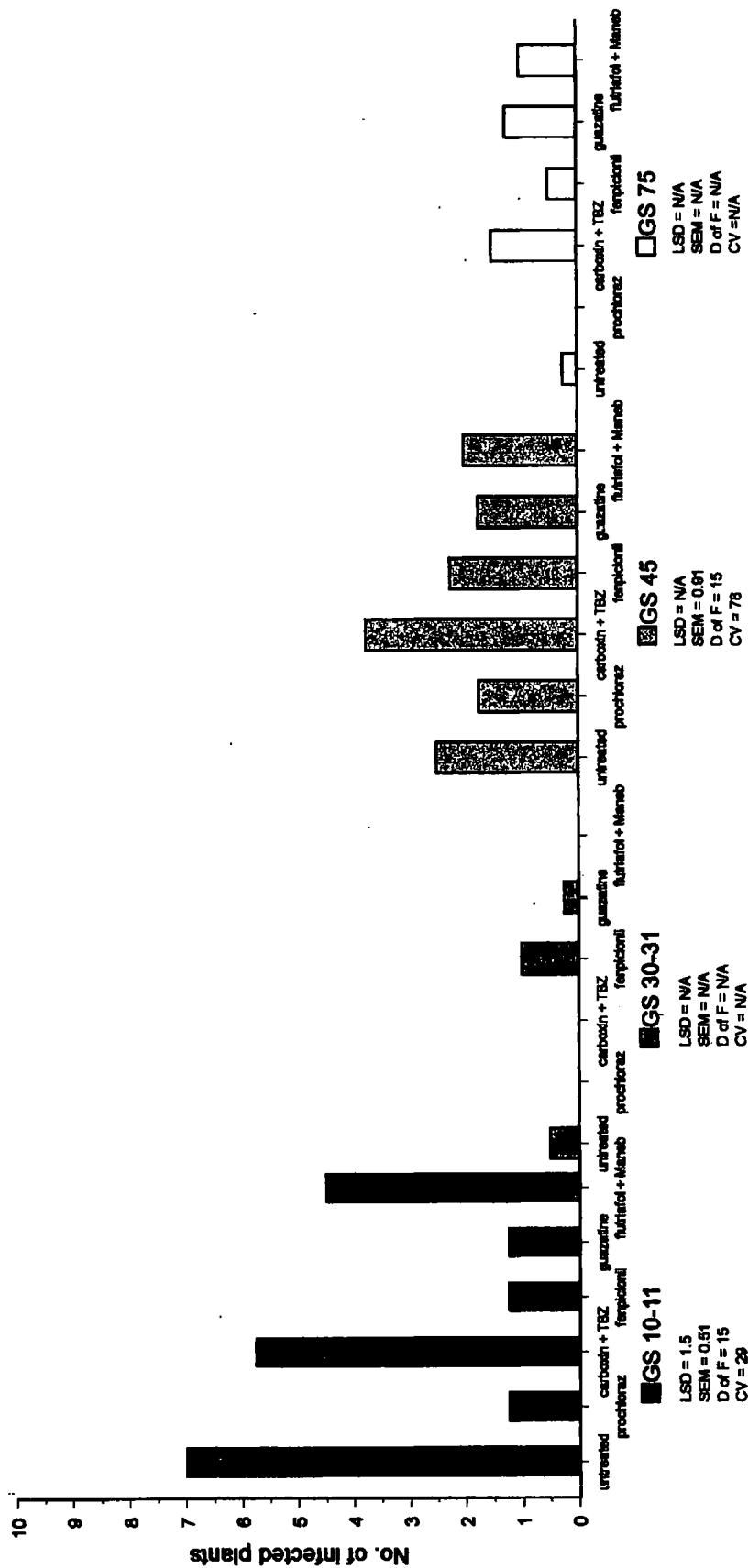


Figure 41 Number of wheat plants out of ten infected by *Microdochium nivale* following treatment with fungicide seed treatments at four times during the growing season (GS 10-11, 30-31, 45 and 75) at trial site No. 1 (for details of site, see Table 24)

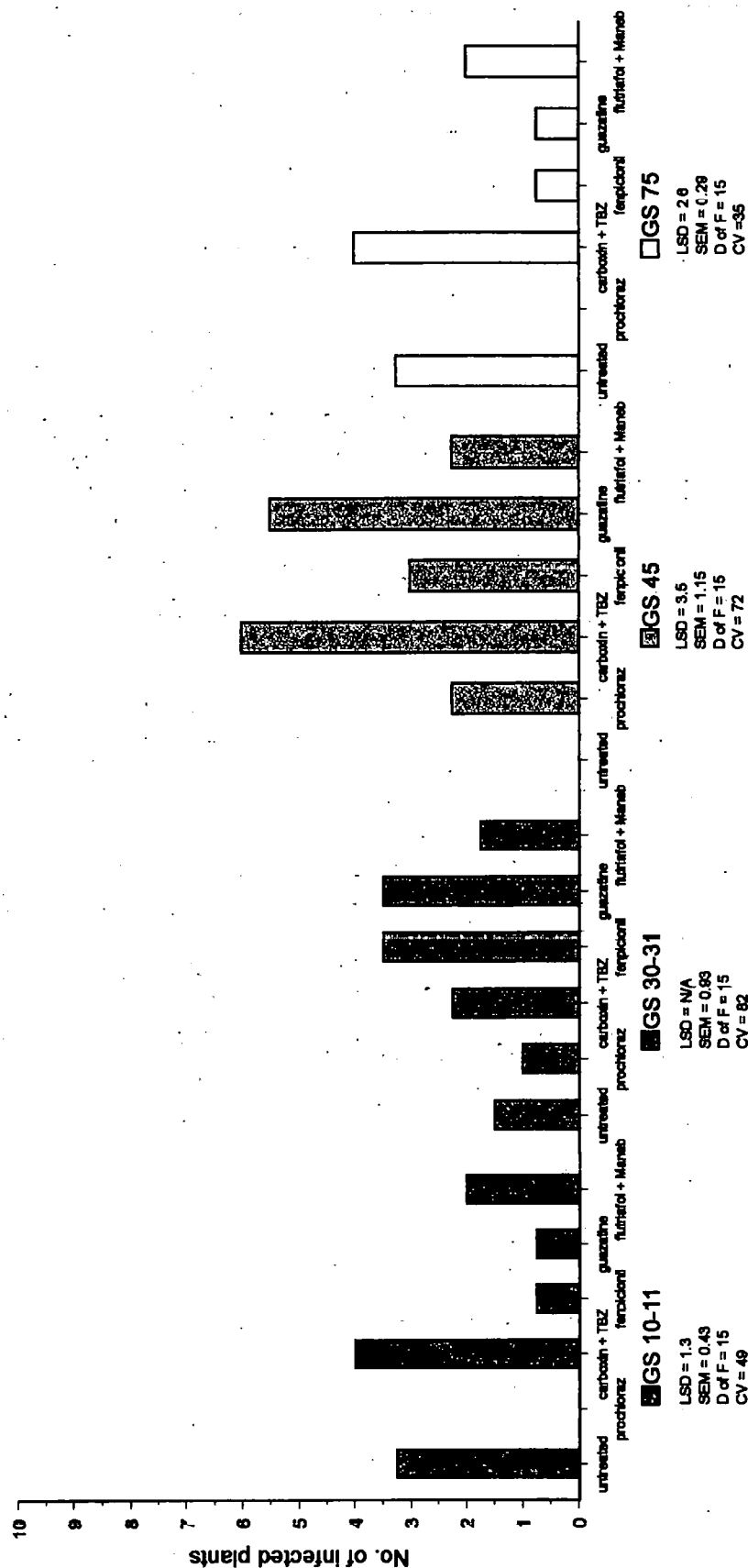


Figure 42 Number of wheat plants out of ten infected by *Microdochium nivale* following treatment with fungicide seed treatments at four times during the growing season (GS 10-11, 30-31, 45 and 75) at trial site No. 2 (for details of site, see Table 24)

However, *F. culmorum* was detected in untreated and guazatine treated plots (0.2 plants) at GS 30-31. The frequency of *F. culmorum* isolations then increased at the latter growth stages to a maximum of 2.8 plants infected in prochloraz treated plots at GS 75, although there were no significant ($P = 0.05$) differences between treatments (Figure 43).

As at site No. 2, no *F. culmorum* stem-base infection was observed at GS 10-11. However, stem-base infection by *F. culmorum* was detected at GS 30-31. Significant differences ($P = 0.05$) between treatments were observed at this growth stage with the greatest number of infected stem-bases occurring in untreated (4.0) and prochloraz (4.2) treated plots. Significantly fewer stem-bases were infected with *F. culmorum* in fenpiclonil (0.5) and guazatine (1.5) plots (Figure 44).

DISCUSSION

The effectiveness of fungicide seed treatments in controlling *Fusarium* seedling blight of wheat has been demonstrated in the field by many workers. However, the robustness of many fungicides is not well understood. Noon and Jackson (1992) sowed a single seed lot of winter wheat naturally contaminated by *M. nivale* and treated with and without a range of fungicide seed treatments at two different field sites, Lincolnshire and Warwickshire. Seedling disease was observed at both sites but was most severe in Lincolnshire. Greater percentage control of seedling disease was observed from the seed treatments in Lincolnshire but final

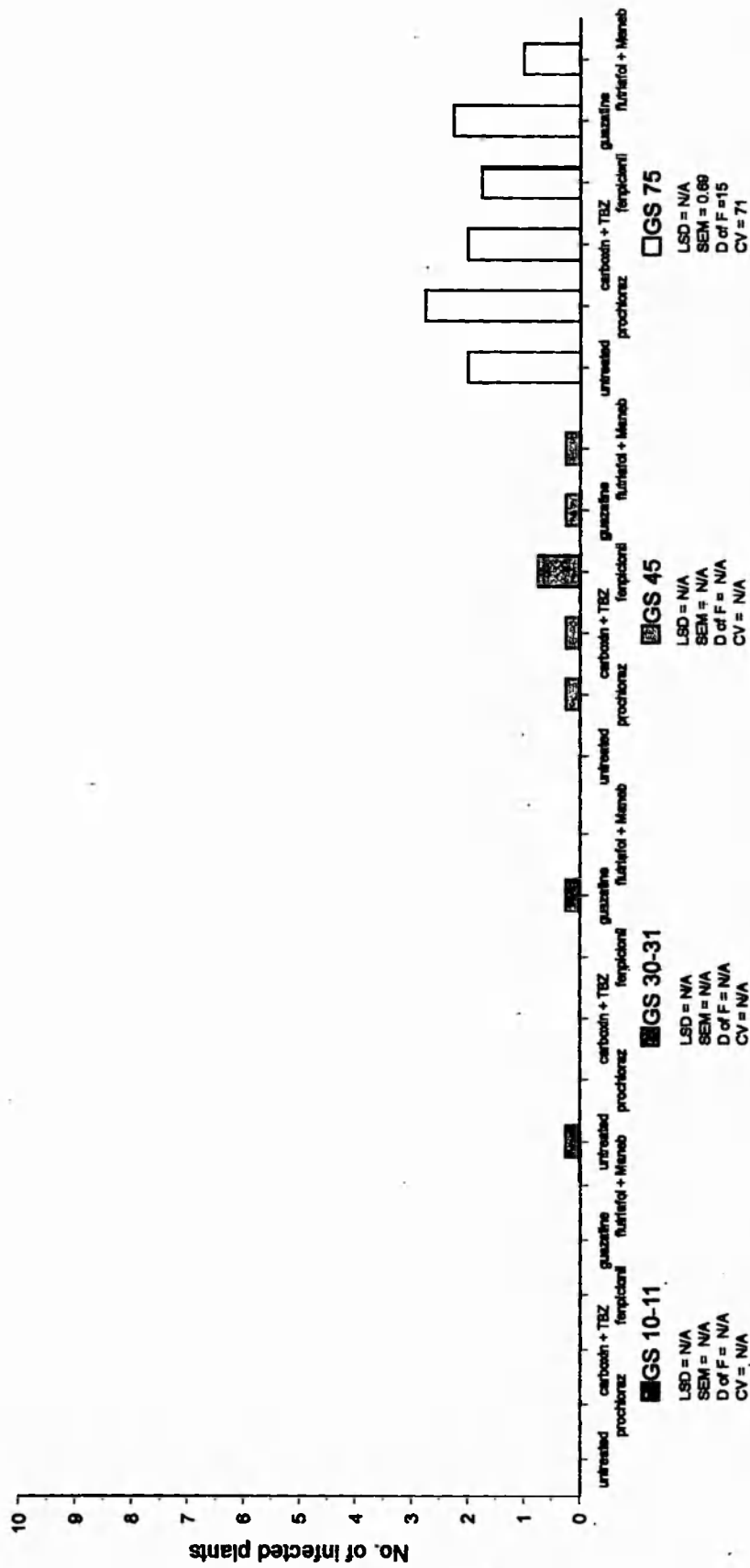
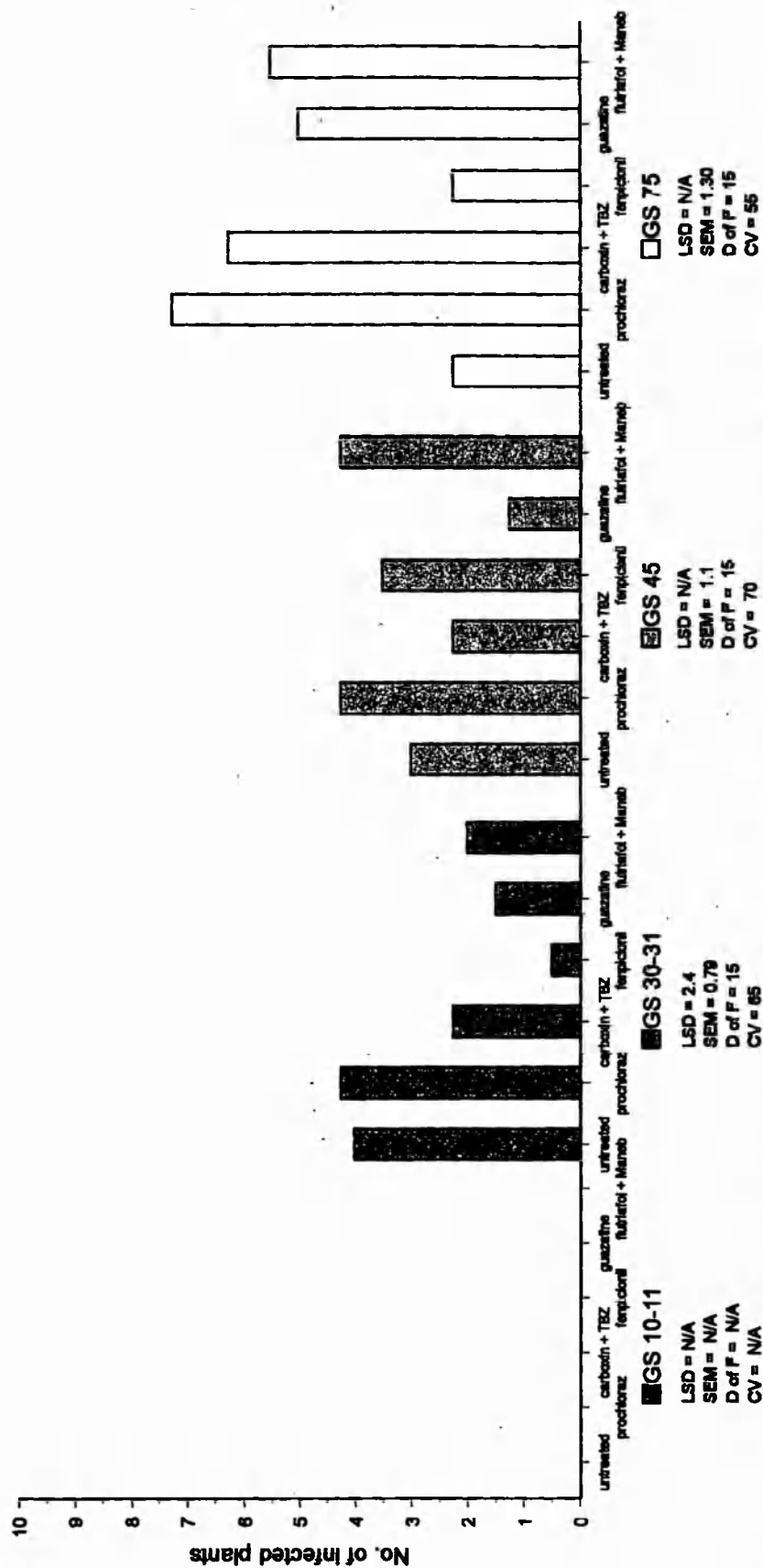


Figure 43 Number of wheat plants out of ten infected by *Fusarium culmorum* following treatment with fungicide seed treatments at four times during the growing season (GS 10-11, 30-31, 45 and 75) at trial site No. 1 (for detail of site, see Table 24)



yield, including the non-treated seed, was greater at the other site in Warwickshire. It is understood that environmental conditions in the seedbed can affect the establishment of non-contaminated wheat seed (Khah *et al.*, 1986), but the effect of the interaction between the host, pathogen, environment and seed treatment on crop establishment and disease severity is not fully documented.

The results of this study from controlled environment experiments showed that the percentage of seeds contaminated by *M. nivale*, of which the majority of isolates were probably *M. nivale* var. *majus* (Table 18), had little effect on the efficacy of the seed treatments tested. This was also seen in the field where similar plant stands were observed in treated plots from two seed lots with different percentage *M. nivale* contaminations. However, a different situation was seen with respect to the effect of temperature on the efficacy of the seed treatments tested.

Guazatine and prochloraz proved to be robust treatments giving similar control of pre-emergence seedling death at each of the temperatures tested whilst treatment with TBZ was ineffective. Control of pre-emergence seedling death was seen from flutriafol although the number of emergent seedlings decreased with decreasing temperature. In warm soil (12°C) good control was achieved but at 6°C seedling emergence had decreased by over 20%. This suggests that flutriafol when used alone as a seed treatment, may not be suitable for the control of *M. nivale* in the field when contaminated seed is sown into cold seedbed conditions.

As seen in previous chapters, there is a good relationship between the rate of seedling emergence and the pre-emergence death of seedlings from naturally

contaminated seed. Of the fungicides tested the most effective were fenpiclonil, guazatine and prochloraz and of these fenpiclonil and guazatine increased the rate of seedling emergence whilst with prochloraz the rate was reduced. There was therefore no clear relationship between the rate of seedling emergence and subsequent disease severity following fungicide treatment owing to the possible negative effect of fungicide treatment on the growth of the plant (Burden *et al.*, 1987).

Microdochium nivale infection affected seedling growth reducing coleoptile, shoot and root length. Where seed treatments were effective at controlling *M. nivale*, coleoptile length and root length of seedlings from treated seeds were longer than the untreated, with the exception of the azole fungicide treatments. Treatment with prochloraz, and to a lesser extent flutriafol, produced seedlings with shorter coleoptiles and roots than other seed treatments, even though disease severity was reduced. The reduced seedling growth seen from these treatments may be attributed to the direct effect of the chemical on the growth of the seedling (Burden *et al.*, 1987), rather than that of the pathogen.

Isolations from established plants at both trial sites indicated that seed-borne *M. nivale* acted as a source of inoculum for stem-base infection and that these infections can initially be reduced by fungicide seed treatments. Similar control of seedling infection from seed treatments was seen by Hutcheon and Jordan (1992) using soil-borne inoculum. Following treatment with a range of fungicides, Hutcheon and Jordan sowed wheat seeds in soil trays to which a mixture of straw colonised by *F. avenaceum*, *F. culmorum*, *F. graminearum* and *M. nivale* was added. They

observed no effect from seed treatment on seedling establishment. However, treatment with flutriafol + TBZ, guazatine and carboxin + TBZ resulted in fewer infected tillers at GS 30 and GS 31.

The duration of the effect of fungicide seed treatments on the control of stem-base infection by *F. culmorum* was studied by Bateman (1980). Bateman showed that of the fungicides tested only the systemic treatments benomyl, carbendazim, TBZ and thiophanate-methyl gave control of *F. culmorum* when wheat seedlings were inoculated 28 days after sowing. The non-systemic fungicide guazatine gave no control of seedling blight symptoms. The control of stem-base infection at GS 10-11 by guazatine at both trial sites in this study may therefore be attributed to disinfection of the seed hence reducing pathogen inoculum rather than the effect of systemic action.

The pattern of *M. nivale* stem-base infection during the growing season was not clear but appeared to be influenced by site and seed treatment, although this was not fully substantiated statistically. At site No. 1 infection declined following assessment at GS 10-11 with few isolates being recovered at GS 30-31. Infection increased again at GS 45 before decreasing at GS 75. At site No. 2 no obvious decline in infection subsequent to GS 10-11 was observed. Stem-base infection increased to a peak at GS 45 before declining again. Where seed treatments reduced stem-base infection at GS 10-11, infection remained low throughout the season.

The reduction in *M. nivale* infection observed at GS 30-31 at site No. 1 followed by an increase in infection later in the season was similar to that seen by

Miedaner *et al.* (1995) in winter rye in Germany. These workers inoculated plants at GS 13 and monitored infection during the season by measuring the presence of fungal protein using enzyme-linked immunosorbent assay (ELISA) within the stem-base. They showed that the greatest amount of fungal protein was present at GS 21 and that the amount of protein decreased significantly during stem extension. The amount of protein then increased from GS 65 to maturity.

Miedaner *et al.* (1995) performed a similar experiment using *F. culmorum* and observed a continuous increase in fungal protein during the season. This observation is very similar to those observed for *F. culmorum* infection at both of the trial sites in the study reported here. No *F. culmorum* was isolated at GS 10-11 at either site however subsequent assessments showed an increase in the number of plants infected.

These patterns of *M. nivale* and *F. culmorum* stem-base infection during the growing season matched closely those observed by Parry (1990) in commercial winter wheat crops. Parry isolated *Fusarium* spp. including *M. nivale* from stem-bases of three wheat crops in each of three years and revealed some interesting trends. In some crops Parry showed that the frequency of *M. nivale* infection dipped during the season in a similar fashion to that seen during this study at sites Nos 1 and 2. He attributed this reduction in infection to either the action of foliar fungicides, for which there was no clear evidence, or the reduction in late-formed tillers and the natural death of stem-base leaf sheaths. Parry cited work by G.L. Bateman received by personal communication in evidence of the latter.

The occurrence of stem-base infection by *F. culmorum* observed by Parry also

followed a similar pattern to those seen at sites Nos 1 and 2 where the frequency of infected plants increased progressively through to GS 75.

CHAPTER 9

General Discussion

DISCUSSION

Fusarium seedling blight is an important disease of wheat in the U.K., not only because it can result in large yield reductions following severe pre-emergence seedling death, but also because it could provide a source of inoculum for subsequent foot rot disease or ear blight epidemics.

Before 1992 and the withdrawal of mercury seed treatments in the U.K. *Fusarium* seedling blight was not considered a serious problem. Mercury seed treatments which were routinely applied to wheat seed gave adequate control of the disease and serious outbreaks of *Fusarium* seedling blight were unknown. However, the importance of *Fusarium* seedling blight as a disease of wheat was re-established when, following the withdrawal of mercury seed treatments in Ireland, severe outbreaks of *Fusarium* seedling blight were seen in many wheat crops.

The withdrawal of mercury based seed treatments in the U.K. resulted in the loss of an inexpensive but effective method of controlling seedling blight. Greater knowledge of the epidemiology and control of *Fusarium* seedling blight was therefore required so that the disease could be managed more effectively.

Source of Inoculum

It has been shown that *M. nivale* can be seed or soil-borne and that these two sources of inoculum can cause seedling blight. Soil-borne inoculum is not thought to

be important as a source of inoculum for seedling blight in U.K. field soils (Paveley and Davies, 1994 and Paveley *et al.*, 1996). Seed-borne *M. nivale* is considered to be the primary source of inoculum for seedling blight (Rennie, 1993), although amounts on seed are variable from year to year (Cockerell and Rennie, 1996).

The contamination of wheat seed by *M. nivale* is a result of ear blight in the preceding seed crop and ear blight epidemics are affected by weather conditions at anthesis (Parry *et al.*, 1995). In the presence of sufficient inoculum ear blight is most severe in cool wet conditions (Rennie and Cockerell, 1993). The occurrence of such conditions are however infrequent and the accurate prediction of seed contamination based on weather data is not yet possible. For these reasons it would be advisable to examine seed for *M. nivale* contamination before sowing. Visual assessment of seed contamination by some fungal pathogens is possible. For example, seed contaminated by *F. culmorum* produces pink, shrivelled seeds and *Alternaria* spp. exhibit dark patches known as "blackpoint". However, visual assessment for *M. nivale* contamination did not prove to be a reliable method for estimating seed contamination in the study reported here. No clear or constant factor was correlated with *M. nivale* contamination (Chapter 3).

Two common methods used for the detection of *M. nivale* in wheat seed are the paper towel and agar plate methods. These methods were evaluated in the study reported here and both proved to be equally effective at identifying *M. nivale* contaminated seeds (Chapter 3). The above methods produce a value for the degree of contamination of a seed lot in terms of the percentage of seeds contaminated by *M.*

nivale but no information regarding the extent of contamination of individual seeds is given. It has been shown that the inoculum load placed on individual seeds can have a significant effect on subsequent disease symptoms (Millar and Colhoun, 1969). However, the percentage of contaminated seeds in a seed lot was shown by Humphreys *et al.* (1995) to be linearly related to seedling establishment. In the study reported here a close linear relationship between percentage *M. nivale* contamination and seedling emergence was not fully established. In general, seedling establishment did decrease with increasing contamination, but there was evidence to suggest that this was not always the case (Chapter 6). In addition, the relationship between percentage contamination and seedling emergence can be affected by environmental conditions (Chapter 6)

Two sub-groups of *M. nivale*: *M. nivale* var *nivale* and *M. nivale* var. *majus* have been identified although little has been written about their relative occurrence on wheat seed or their pathogenicity towards wheat seedlings. In the study reported here it was shown that *M. nivale* var. *majus* was the predominant sub-group on the wheat seed samples tested. Of 91 *M. nivale* isolates recovered from seven seed lots of winter wheat 85 were *M. nivale* var. *majus* and six were *M. nivale* var *nivale*. The pathogenicity of the two sub-groups was investigated and all isolates tested produced disease symptoms in wheat seedlings, although isolates of *M. nivale* var. *majus* produced the most severe symptoms especially with respect to pre-emergence seedling death (Chapter 7).

The Effects of Temperature and Soil Water on *Fusarium* Seedling Blight

Previous workers have investigated the effects of temperature and soil water on the severity of *Fusarium* seedling blight. It is generally understood that seedling blight symptoms of wheat following artificial inoculation with *F. culmorum* are most severe in warm, dry soils (Colhoun and Park, 1964), whereas those from *M. nivale* are most severe in cold, dry soils (Millar and Colhoun, 1969). This was supported by the study reported here which, in addition, showed that seedling disease caused by natural *M. nivale* contamination was also most severe in cold, dry soil (Chapter 5).

The effect of temperature and soil water on the rate of wheat seedling emergence and the relationship between the rate of emergence and subsequent seedling disease caused by *M. nivale* was substantiated in the study reported here (Chapters 5 and 6). The rate of seedling emergence was reduced in cold, dry soil conditions and there was a close linear relationship between the rate of seedling emergence and the number of emerged seedlings, both from artificially inoculated and naturally contaminated seeds.

Control of *Fusarium* Seedling Blight of Wheat

The fact that wheat seed contaminated by *Fusarium* spp. are often small, light and shrivelled was used by Nakagawa and Yamaguchi (1989) to remove seed contaminated by *F. roseum* from a seed lot of winter wheat. They floated off the

small shrivelled contaminated seeds from the non-contaminated seeds in a saline solution, producing a seed sample with fewer contaminated seeds than the original seed lot. In the study reported here investigation of three seed lots of wheat naturally contaminated by *M. nivale* showed that small light seeds were not the sole contaminated component of the seed lot. Contaminated seeds were evenly distributed by weight throughout each of the seed lots examined. Therefore, unlike *F. roseum*, where the “cleaning” of naturally contaminated seed lots was possible by the removal of small light seeds (Nakagawa and Yamaguchi, 1989), *M. nivale* contaminated seed lots cannot be “cleaned” on the basis of seed size or weight. In addition, when sown, small light seed suffered less than large heavy seed from subsequent pre-emergence seedling death. More seedlings emerged from small light seeds than large seed and this effect was attributed to the faster rate of seedling emergence seen from the small light seeds (Chapter 4).

In the study reported here, most of the fungicide seed treatments known to control *M. nivale* seedling blight of wheat proved to be robust under a range of conditions. The percentage of seeds in a seed lot naturally contaminated by *M. nivale* did not affect the efficacy of the seed treatments tested. However, treatment with flutriafol did not prove to be robust under conditions of decreasing temperature (Chapter 8). In warm soil (12°C) flutriafol worked well and effectively controlled pre-emergence death of seedlings. However at 6°C control was not as good and seedling emergence had decreased by over 20%. Environmental conditions at sowing must therefore be taken into account when choosing a fungicide seed treatment for the

control of *M. nivale* seedling blight of wheat.

Good control of pre-emergence seedling death was observed in the study reported here from fungicide seed treatments in the field. However, not all seedlings that emerged were free from *M. nivale* infection. Seed-borne *M. nivale* appeared to be transmitted to established wheat seedlings and treatment with carboxin + TBZ was the least effective treatment at reducing seedling infection. The effect of seed treatment on early seedling infection by *M. nivale* appeared to be carried through the season with the most effective treatments having fewer infected plants at subsequent assessments. However, this effect was not tested statistically. Stem-base infection by *F. culmorum* was observed later in the season with infection increasing progressively from GS 30-31 to GS 75. This observation is very similar to those of Parry (1990) in the U.K. and Miedaner *et al.* (1995) in Germany.

PROPOSED FURTHER STUDIES

- i) The use of competitive PCR to determine the relationship between the percentage of seeds in a seed lot naturally contaminated by *M. nivale*, the inoculum load on individual seeds, the inoculum load within the seed lot as a whole and subsequent disease severity.
- ii) Further controlled environment studies to investigate the relationship between seed-borne *M. nivale* and the speed of seedling emergence.
- iii) Closely monitored field studies to establish the relationship between *M. nivale* seed contamination, seedbed conditions, the rate of seedling emergence and subsequent disease severity.
- iv) The effect of fungicide seed treatments on the relationship (iii) above.
- v) An extensive survey of the occurrence of *M. nivale* var. *majus* and *M. nivale* var. *nivale* in wheat seed and further investigations into the impact of the two sub-groups on the epidemiology and control of seedling disease.

REFERENCES

REFERENCES

- Al-Hashimi, M.H. and Perry, D.A.** 1986. Survival and saprophytic ability of *Monographella nivalis* in soil. *Transactions of the British Mycological Society*. **86**, 373-379.
- Anon.** 1977a. *Techniques for measuring soil physical properties*. London: Ministry of Agriculture Fisheries and Food, p82.
- Anon.** 1977b. *Techniques for measuring soil physical properties*. London: Ministry of Agriculture Fisheries and Food, pp86-88.
- Anon.** 1979. Council Directive 79/117/EEC of 12 December 1978 prohibiting the placing on the market and use of plant protection products containing certain active substances. *Official Journal of the European Communities*. **22** L33, 8.2. 1979, 36-40.
- Anon.** 1985. International rules for seed testing. *Seed Science and Technology*. **13**, 299-355.
- AOSA.** 1983. *Seed Vigour Testing Handbook*. Association of Official Seed Analysts, USA.
- Armstrong, G.M. and Armstrong, J.K.** 1948. Non-susceptible hosts as carriers of wilt *Fusaria*. *Phytopathology*. **38**, 808-826.
- Bateman, G.L.** 1976. Control of seed-borne *Fusarium nivale* on wheat and barley by organo-mercury seed treatment. *Annals of Applied Biology*. **83**, 245-250.
- Bateman, G.L.** 1977. Effects of organo-mercury treatment of wheat seed on *Fusarium* seedling disease in inoculated soil. *Annals of Applied Biology*. **85**, 195-201.
- Bateman, G.L.** 1980. Uptake and translocation of fungicides in wheat after seed treatment as measured by disease response to *Fusarium culmorum*. *Pesticide Science*. **11**, 651-659.
- Bateman, G.L.** 1983. Response of *Fusarium nivale* in wheat and barley caryopses to organomercury treatment in relation to site of infection and microbial antagonism. *Transactions of the British Mycological Society*. **81** (1), 141-183.

- Bechtel, D.B., Kaleikau, L.A., Gaines, R.L. and Seitz, L.M.** 1985. The effects of *Fusarium graminearum* infection on wheat kernels. *Cereal Chemistry*. **62**, 191-197.
- Bennett, F.T.** 1928. On two species of *Fusarium*, *F. culmorum* (W.G.Sm.) Sacc. and *F. avenaceum* (Fries.) Sacc, as parasites of cereals. *Annals of Applied Biology*. **15**, 213-244.
- Bennett, F.T.** 1935. *Fusarium* species on British cereals. *Annals of Applied Biology*. **22**, 479-501.
- Blair, L.D.** 1937. An investigation of foot rot of wheat in New Zealand. *New Zealand Journal of Science and Technology*. **19**, 1.
- Booth, C.** 1971. The Genus *Fusarium*. The Commonwealth Mycological Institute, Kew, Surrey.
- Booth, R.H. and Taylor, G.S.** 1976a. *Fusarium* diseases of cereals X. Straw debris as a source of inoculum for infection of wheat by *Fusarium nivale* in the field. *Transactions of the British Mycological Society*. **66** (1), 71-75.
- Booth, R.H. and Taylor, G.S.** 1976b. *Fusarium* diseases of cereals XI. Growth and saprophytic activity of *Fusarium nivale* in soil. *Transactions of the British Mycological Society*. **66** (1), 77-83.
- Burgess, L.W., Klein, T.A., Bryden, W.L. and Tobin, N.F.** 1987. Head blight of wheat caused by *Fusarium graminearum* Group 1 in NSW in 1983. *Australian Journal of Plant Pathology*. **16**, 72-78
- Burden, R.S., Carter, G.A., Clark, T., Cooke, D.T., Croker, S.J., Deas, A.H.B., Hedden, P., James, C.S. and Lenton, J.R.** 1987. Comparative activity of the enantiomers of triadimenol and paclobutrazol as inhibitors of fungal growth and plant sterol and gibberellin biosynthesis. *Pesticide Science*. **21**, 253-267.
- Cariddi, C.** 1982. Infezione di *Fusarium nivale* (Fr.) Ces. in cariossidi di Frumento e moria dei seminati. *Informatore Fitopatologico*. **82**, 59-60.
- Cassell, D. and Hering, T.F.** 1982. The effects of water potential on soil-borne diseases of wheat seedlings. *Annals of Applied Biology*. **101**, 367-375.

- Cassini, R.** 1981. *Fusarium* diseases of cereals in Western Europe. In: Nelson, P.E., Toussoun, T.A. and Cook R.J. eds. *Fusarium: Diseases, Biology and Taxonomy*. University Park and London: The Pennsylvania State University Press. pp 58-63.
- Clark, M.R.M.** 1981. The effects of organo-mercury seed treatment on barley artificially inoculated with *Fusarium* spp. EPPO Bulletin. 11 (3), 317-322.
- Clear, R.M. and Patrick, S.K.** 1990. *Fusarium* species isolated from wheat samples containing tombstone (scab) kernels from Ontario, Manitoba and Saskatchewan. *Canadian Journal of Plant Science*. 70, 1057-1069.
- Cockerell, V.** 1995. Review of work carried out at the Official Seed Testing Station (OSTS) on the control of *Microdochium nivale* (*Fusarium nivale*) on winter wheat in Scotland. *Proceedings of the ISTA Pre-Congress Seminar. Seed health testing and the production of quality seed*. Copenhagen, 6 June 1995, pp 25-38.
- Cockerell, V. and Rennie W.J.** 1996. Survey of seed-borne pathogens in certified and farm-saved cereal seed in Britain between 1992 and 1994. *Home-Grown Cereals Authority Project Report No. 124*. London: Home-Grown Cereals Authority.
- Colhoun, J.** 1970. Epidemiology of seed-borne *Fusarium* diseases of cereals. *Annales Academie Scientiarum Fennicae*. 186, 31-35.
- Colhoun, J. and Park, D.** 1964. *Fusarium* diseases of cereals I Infection of wheat plants, with particular reference to the effects of soil moisture and temperature on seedling infection. *Transactions of the British Mycological Society*. 47 (4), 559-572.
- Colhoun, J., Taylor, G.S. and Tomlinson, R.** 1968. *Fusarium* diseases of cereals II. Infection of seedlings by *Fusarium culmorum* and *Fusarium avenaceum* in relation to environmental factors. *Transactions of the British Mycological Society*. 51, 397-404.
- Cook, R.J.** 1968. *Fusarium* root and foot rot of cereals in the Pacific Northwest. *Phytopathology* 58, 127-131
- Cook, R.J.** 1980. *Fusarium* foot rot of wheat and its control in the Pacific Northwest. *Plant Disease*. 64, 1061-1066.

- Cristani, C.** 1992. Seed-borne *Microdochium nivale* (Ces. ex Sacc.) Samuels (= *Fusarium nivale* (Fr.) Ces.) in naturally infected seeds of wheat and triticale in Italy. *Seed Science and Technology*. **20**, 603-617.
- Daamen, R.A., Langerak, C.J. and Stol, W.** 1991. Surveys of cereal pests and diseases in the Netherlands 3. *Monographella nivalis* and *Fusarium* species in winter wheat fields and seed lots. *Netherlands Journal of Plant Pathology*. **97**, 105-114.
- De Tempe, J. and Limonard, T.** 1973. Seed-fungal-bacterial interactions. *Seed Science and Technology*. **1**, 203-216.
- Dickson, J.G.** 1921. Influence of soil temperature and soil moisture on the development of the seedling blight of winter wheat and corn caused by *Gibberella saubineti*. *Journal of Agricultural Research*. **23** (11), 837-870.
- Dickson, J.G., Johann, H. and Wineland, G.** 1921. Second progress report of the *Fusarium* blight (scab) of wheat. *Phytopathology*. **11**, 35-36.
- Duben, J. and Fehrman, H.** 1979. Occurrence and pathogenicity of *Fusarium* species on winter wheat in West Germany II. Comparison of pathogenicity on seedlings, haulm bases and ears. *Journal of Plant Diseases and Protection*. **86** (12), 705-728.
- Duthie, J.A. and Hall, R.** 1987. Transmission of *Fusarium graminearum* from seed to stems of winter wheat. *Plant Pathology*. **36**, 33-37.
- Elekes, P.** 1983. Methods for detecting hyphal and living infection in wheat seed samples. *Seed Science and Technology*. **11**, 421-433.
- Francis, R.G. and Burgess, L.W.** 1977. Characteristics of two populations of *Fusarium roseum* "Graminearum" in Eastern Australia. *Transactions of the British Mycological Society* **68**, 421-427.
- Frohberger, P.E.** 1978. Baytan, a new systemic broad spectrum fungicide especially suitable for cereal seed treatment. *Pflanzenschutz-Nachrichten Bayer*. **31**, 11.
- Fukui, R., Campbell, G. S. and Cook, R. J.** 1994. Factors influencing the incidence of embryo infection by *Pythium* spp. during germination of wheat seeds in soils. *Phytopathology* **84**, 695-702.

- Gerlach, W. and Nirenberg, H.** 1982. The genus *Fusarium*, a Pictorial Atlas. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Fortwirtschaft, Berlin-Dahlem.* 209, pp406.
- Gordon, W.L.** 1954. The occurrence of *Fusarium* species in Canada III. Taxonomy of *Fusarium* species in the seed of vegetable, forage and miscellaneous crops. *Canadian Journal of Botany.* 32, 576-590.
- Gordon, W.L.** 1959. The occurrence of *Fusarium* species in Canada VI. Taxonomy and geographic distribution of *Fusarium* species on plants, insects and fungi. *Canadian Journal of Botany.* 37, 257-290.
- Grabarkiewicz-Szczesna, J. and Chelkowski, J.** 1993. Occurrence of *Alternaria* mycotoxins in wheat and triticale grain with "black point" injuries. *Microbiologie - Aliments - nutrition.* 11, 183-185.
- Griffin, D.M.** 1969. Soil water and the ecology of fungi. *Annual Review of Phytopathology.* 7, 289-310.
- Hargreaves, A.J. and Fox, R.A.** 1978. Some factors affecting survival of *Fusarium avenaceum* in soil. *Transactions of the British Mycological Society.* 70 (2), 209-212.
- Hewett, P.D.** (1965) A survey of seed-borne fungi of wheat. The incidence of *Leptosphaeria nodorum* and *Griphosphaeria nivalis*. *Transactions of the British Mycological Society.* 48, 59-72.
- Hewett, P.D.** 1967. A survey of seed-borne fungi of wheat II. The incidence of common species of *Fusarium*. *Transactions of the British Mycological Society.* 50 (2), 175-182.
- Hewett, P.D.** 1983. Seed-borne *Gerlachia nivalis* (*Fusarium nivale*) and reduced establishment of winter wheat. *Transactions of the British Mycological Society.* 80 (1), 185-186.
- Humphreys, J., Cooke, B.M. and Storey, T.** 1995. Effects of seed-borne *Microdochium nivale* on establishment and grain yield of winter-sown wheat. *Plant Varieties and Seeds.* 8, 107-117.
- Hutcheon, J.A. and Jordan, V.W.L.** 1992. Fungicide timing and performance for *Fusarium* control in wheat. *Brighton Crop Protection Conference. Pests and Diseases.* 633-638.

- Hyde, M.B. 1950. The sub-epidermal fungi of cereal grains I. A survey of the world distribution of fungal mycelium in wheat. *Annals of Applied Biology*. 37, 179-186.
- Hyde, M.B. and Galleymore, H.B. 1951. The sub-epidermal fungi of cereal grains. II. The nature, identity and origin of the mycelium in wheat. *Annals of Applied Biology*. 38 (2), 348-358.
- Jackson, D., Roscoe, R.J. and Ballard, N.E. 1973. The effectiveness of Bis (8-guanadinocetyl) amine sulphate, a new broad spectrum fungicide especially effective against seed-borne diseases of cereals. *First International Congress Plant Pathology 1968* p27 (abstr).
- Jackson, D., Marshall, R., Tomkins, M.J., Novy, O., Papp, Z. and Romaniuk, J. 1994. The role of carboxin + thiram FS in post-mercury seed treatment fungicide strategies in Central Europe. In: Martin, T. ed. *Seed treatment: progress and prospects*. BCPC Monograph No. 57. 123-128.
- Jenkinson, P. and Parry, D.W. 1994. Splash dispersal of conidia of *Fusarium culmorum* and *Fusarium avenaceum*. *Mycological Research*. 98, 506-510.
- Kelman, A. and Cook, R.J. 1977. Plant Pathology in The Peoples Republic of China. *Annual Review of Phytopathology*. 17, 409-429.
- Khah, E.M., Ellis, R.H. and Roberts, E.H. 1986. Effects of laboratory germination, soil temperature and moisture content on the emergence of spring wheat. *Journal of Agricultural Science, Cambridge*. 107, 431-438.
- Klein, T.A. and Burgess, L.W. 1987. Effect of seed treatment on infection of wheat by seed-borne inoculum of *Fusarium graminearum* group 1. *Australasian Plant Pathology*. 16 (4), 79-81.
- Koch, E. and Leadbeater, A.J. 1992. Phenylpyrroles - A new class of fungicides for seed treatment. *Brighton Crop Protection Conference - Pests and Diseases-1992*. 9B-3, 1137-1146.
- Lafond, G.P. and Baker, R.J. 1986a. Effects of genotype and seed size on speed of emergence and seedling vigour in nine spring wheat cultivars. *Crop Science*. 26, 341-346.
- Lafond, G.P. and Baker, R.J. 1986b. Effects of temperature, moisture stress, and seed size on germination of nine spring wheat cultivars. *Crop Science*. 26, 563-567.

- Leach, L.D.** 1947. Growth rates of host and pathogen as factors determining the severity of pre-emergence damping-off. *Journal of Agricultural Research*. **75**, 161-179.
- Lees, A.K., Nicholson, P., Rezanoor, H.N. and Parry, D.W.** 1995. Analysis of variation within *Microdochium nivale* from wheat: evidence for a distinct sub-group. *Mycological research*. **99**, 103-109
- Liddell, C.M. and Burgess, L.W.** 1988. Wax partitioned soil columns to study the influence of soil moisture potential on the infection of wheat by *Fusarium graminearum* Group 1. *Phytopathology* **78**, 185-189.
- Limonard, T.** 1968. Ecological aspects of seed health testing. *Proceedings of the International Seed Testing Association*. **33**, 343-511.
- Litschko, L. and Burpee, L.L.** 1987. Variation among isolates of *Microdochium nivale* collected from wheat and turf grasses. *Transactions of the British Mycological Society*. **89**, 252-256
- Locke, T., Moon, L.M. and Evans, J.** 1987. Survey of benomyl resistance in *Fusarium* species on winter wheat in England and Wales 1986. *Plant Pathology*. **36**, 589-593.
- Machacek, J.E. and Greaney, F.J.** 1935. Studies on the control of root rot diseases of cereals caused by *Fusarium culmorum* (W.G.Sm.) Sacc. and *Helminthosporium sativum* P., K., and B. III. Effect of seed treatment on the control of root rot and on the yield of wheat. *Scientific Agriculture*. **15**, 607-629.
- Malalasekera, R.A.P. and Colhoun, J.** 1968. *Fusarium* diseases of cereals III. Water relations and infection of wheat seedlings by *Fusarium culmorum*. *Transactions of the British Mycological Society*. **51** (5), 711-720.
- Malalasekera, R.A.P. and Colhoun, J.** 1969 *Fusarium* diseases of cereals V. A technique for the examination of wheat seed infected with *Fusarium culmorum*. *Transactions of the British Mycological Society*. **52** (2), 187-193.
- Malalasekera, R.A.P., Sanderson, F.R. and Colhoun, J.** 1973. *Fusarium* diseases of cereals IX. Penetration and invasion of wheat seedlings by *Fusarium culmorum* and *Fusarium nivale*. *Transactions of the British Mycological Society*. **60** (3), 435-462.

- Malan, G.E.** 1949. Azione disgregatrice del *Fusarium nivale* sulle foglie di segale in natura. *Nuovo Giornale Botanico Italiano*. **56**, 435-462. (Abstract in *Review of Applied Mycology*. **30**, 33-34. 1951.).
- Maric, A.** 1981. Fusarium diseases of wheat and corn in Eastern Europe and the Soviet Union. In: **Nelson, P.E., Toussoun, T.A. and Cook R.J. eds.** *Fusarium: Diseases, Biology and Taxonomy*. University Park and London: The Pennsylvania State University Press. pp 77-93.
- Maurin, N.** 1993. Characterization of 31 strains of *Microdochium nivale* (ex *Fusarium nivale*). Abstracts of the 6th International Congress of Plant Pathology.
- Maurin, N., Rezanoor, H.N., Lamkadmi, Z., Somè, A. and Nicholson, P.** 1995 A comparison of morphology, pathogenicity, peritheciium formation, isozyme and DNA markers for the analysis of variability within *Microdochium nivale* (Fries) Samuels and Hallet. *Agronomie*. **15**, 39-47.
- Miedaner, T., Beyer, W., Höxter, H. and Geiger, H.H.** 1995. Growth stage specific resistance of winter rye to *Microdochium nivale* and *Fusarium* species in the field assessed by immunological methods. *Phytopathology*. **85** (4), 416-421.
- Mihuta-Grimm, L. and Forster, R.L.** 1989. Scab of wheat and barley in Southern Idaho and evaluation of seed treatments for eradication of *Fusarium* spp. *Plant Disease*. **73** (9), 769-771.
- Millar, C.S. and Colhoun, J.** 1969. *Fusarium* diseases of cereals IV. Observations on *Fusarium nivale* on wheat. *Transactions of the British Mycological Society*. **52** (1), 57-66.
- Morris, D.B., Wainwright, A. and Meredith, R.H.** 1994. New seed treatments based on bitertanol and tebuconazole for seed-borne disease control in wheat. In: **Martin, T. ed.** *Seed treatment: progress and prospects*. BCPC Monograph No. 57. 97-102.
- Nakagawa, A. and Yamaguchi, T.** 1989. Seed treatment for control of seed-borne *Fusarium roseum* on wheat. *Japan Agricultural Research Quarterly*. **23** (2), 94-99.
- Neergaard, P.** 1977. *Seed Pathology, volumes I and II*. London: The Macmillan Press.

- Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. 1983. *Fusarium species, an illustrated manual for identification*. University Park and London: The Pennsylvania State University Press. pp193.
- Noon, R.A. and Jackson, D. 1992. Alternatives to mercury for control of cereal seed-borne diseases. Brighton Crop Protection Conference. Pests and Diseases. 1127-1136.
- Oyarzun, P. J., Van Der Zalm, T., Roelofsen, H.W. and Dijst, G. 1994
Computerized equipment to control soil water matric potential and soil temperature in inoculum potential assays of soil-borne pathogens. *Plant and Soil*. 166, 125-135.
- Parry, D.W. 1990. The incidence of *Fusarium* spp. in stem bases of selected crops of winter wheat in the Midlands, UK. *Plant Pathology*. 39, 619-622
- Parry, D.W., Pettitt, T.R., Jenkinson, P. and Lees, A.K. 1994. The cereal *Fusarium* complex. In: Blakeman, P. and Williamson, B. eds. *Ecology of plant pathogens*. Wallingford, UK: CAB International, 301-320.
- Parry, D.W., Jenkinson, P. and McLeod, L. 1995. *Fusarium* ear blight (scab) in small grain cereals - a review. *Plant Pathology*. 44 (2), 207-238.
- Parry, D.W., Rezanoor, H.N., Pettitt, T.R., Hare, M.C. and Nicholson, P. 1995. Analysis of *Microdochium nivale* isolates from wheat in the UK during 1993. *Annals of Applied Biology*. 126, 449-455.
- Paveley, N.D. and Davies, J.M.L. 1994. Cereal seed treatment - risks, costs and benefits. In: Martin, T. ed. *Seed Treatment: Progress and Prospects*, BCPC Monograph No. 57. 27-35.
- Paveley, N.D., Rennie, W.J., Reeves, J.C., Wray, M.W., Slawson, D.D., Clark, W.S., Cockerell, V. and Mitchell, A.G. 1996. Home-Grown Cereals Authority Research Review No. 34: Cereal Seed Health and Seed Treatment Strategies.
- Perkowski, J. and Chelkowski, J. 1991. Deoxynivalenol content dependence on kernel size in wheat grain naturally infected by *Fusarium*. *Microbiologie - Aliments - Nutrition*. 9, 217-221.
- Perry, D.A. and Al-Hashimi, M. 1983. Epidemiology and etiology of fungal root diseases of cereals. *Report -Scottish Crop Research Institute*. pp94-95.

- Pettitt, T.R., Parry, D.W. and Polley, R.W. 1993. Improved estimation of the incidence of *Microdochium nivale* in winter wheat stems in England and Wales, during 1992, by the use of benomyl agar. *Mycological Research*. 97 (10), 1172-1174.
- Polley, R.W., Turner, J.A., Cockerell, V., Robb, J., Scudamore, K.A., Sanders, M.F. and Magan, N. 1991. *Survey of Fusarium species infecting winter wheat in England, Wales and Scotland, 1989 and 1990*. Home-Grown Cereals Authority. Project report 39.
- Rawlinson, C.J. and Colhoun, J. 1969. The occurrence of *Fusarium nivale* in soil. *Plant Pathology*. 18, 41-45.
- Remy, T. and Vasters, L. 1914. Beobachtungen uber Chlorophenol - Quecksilber als pflanzenschutzmittel. *Illus. Landw. Zty. Jahrg.* 34 (91), 769-770 and (92), 776-778.
- Rennie, W.J., Tomlin, M.M. and Don R. 1980. An examination of some factors affecting the quality of winter wheat seed sown in Scotland in 1976 and 1977. *Journal of the National Institute of Agricultural Botany*. 15, 362-371.
- Rennie, W.J., Richardson, M.J. and Noble, M. 1983. Seed-borne pathogens and the production of quality seed in Scotland. *Seed Science and Technology*. 11, 1115-1127.
- Rennie, W.J. and Gorey, V. 1988. Estimating potential germination of winter wheat and winter barley seed using a tetrazolium test. *Irish Journal of Agricultural Research*. 27, 179-186.
- Rennie, W.J., Cockerell, V., Don, R. and Sommerville, J. 1990. Assessing the germination potential of *Monographella nivalis* infected winter wheat seed. *Proceedings Crop Protection in Northern Britain*. pp101-108.
- Rennie, W.J. 1993. The need for cereal seed treatment in the UK in the post-mercury era. *Pesticide Outlook*. 4, 19-24.
- Rennie, W.J. and Cockerell, V. 1993. A review of seed-borne pathogens of cereals in the post-mercury period. *Proceedings Crop Protection in Northern Britain 1993*. 17-24.
- Richardson, M.J. 1974. Response of oats to seed treatment and seed-borne inocula of *Pyrenophora avenae* and *Micronectriella nivalis*. *Transactions of the British Mycological Society*. 62 (3), 567-584.

- Russell, T.A.** 1931. Observations on foot rot diseases of cereals. *Transactions of the British Mycological Society*. **16**, 253-269.
- Sadasivan, T.S.** 1939. Succession of fungi decomposing wheat straw in different soils, with special reference to *Fusarium culmorum*. *Annals of Applied Biology*. **26**, 497-508.
- Samuels, G.J. and Hallett, I.C.** 1983. *Microdochium stoveri* and *Monographella stoveri*, new combinations for *Fusarium stoveri* and *Micronectriella stoveri*. *Transactions of the British Mycological Society*. **81**, 473-483.
- Scheinflug, H. and Duben, J.** 1988. Experience with novel fungicidal seed treatments for cereals. *Pflanzenschutz-Nachrichten Bayer*. **41**, 258-284.
- Shen, C.I.** 1940. Soil conditions and the *Fusarium culmorum* seedling blight of wheat. *Annals of Applied Biology*. **27**, 323-329.
- Shotwell, O., Bennett, G.A., Stubblefield, R.D., Shanon, G.M., Kwolek, W.F. and Plattner, R.D.** 1985. Deoxynivalenol in hard red winter wheat: relationship between toxin levels and factors that could be used in grading. *Journal of the Association of Official Analytical Chemists*. **68**, 954-957.
- Simmonds, P.M.** 1928. Seedling blight and foot rot of oats caused by *F. culmorum*. Canadian Department of Agriculture, Bulletin No. **105**, 1-43.
- Smith, W.G.** 1884. *Diseases of Field and Garden Crops*. London: MacMillan and Co.
- Smith, J.D.** 1983. *Fusarium nivale* (*Gerlachia nivalis*) from cereals and grasses: is it the same fungus? *Canadian Plant Disease Survey*. **63**, 25-26.
- Snijders, C.H.A.** 1990. Systemic fungal growth of *Fusarium culmorum* in stems of winter wheat. *Journal of Phytopathology*. **129**, 133-140.
- Snijders, C.H.A. and Perkowski, J.** 1990. Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. *Phytopathology*. **80**, 566-570.
- Snyder, W.C. and Nash, S.M.** 1968. Relative incidence of *Fusarium* pathogens of cereals in rotation plots at Rothamsted. *Transactions of the British Mycological Society*. **51**, 417-425.

- Tarr, S.A.J.** 1962. *Diseases of Sorghum, Sudan Grass and Broom Corn*. Commonwealth Mycological Institute, Kew, Surrey, U.K.
- Uoti, J.** 1976. The effect of five *Fusarium* species on the growth and development of spring wheat and barley. *Annales Agriculturae Fenniae*. **15**, 254-262.
- Uoti, J.** 1979. Study of control of seed-borne *Fusarium* in cereals. *Annales Agriculturae Fenniae*. **18**, 149-153.
- Wadleigh, C.H., Gauch, H.G. and Magistad, O.C.** 1946. Growth and rubber accumulation in Guayule as conditioned by soil salinity and irrigation regime. *US Department of Agriculture Technical Bulletin*. 925.
- Wainwright, A., Rollett, A.C. and Morris, D.B.** 1979 Triadimenol seed treatments for the control of cereal diseases. *Brighton Crop Protection Conference. Pests and Diseases*. 565-574.
- Wainwright, A. and Linke, W.** 1987. Field trials with Folicure and Raxil against foliar and seed-borne cereal diseases, and observations on the control of rape diseases with Folicure in Great Britain (1984-1986). *Pflanzenschutz-Nachrichten Bayer*. **2**, 181-212.
- Wakuliński, W. and Chelkowski, J.** 1993. *Fusarium* species transmitted with seed of wheat, rye, barley, oats and triticale. *Hod. Rośl. Aklim. Nasien*. **37** (4), 131-136.
- Walker, A.G.** 1942. The colonization of buried wheat straw by soil fungi, with special reference to *Fusarium culmorum*. *Applied Biology*. **28**, 333-350.
- Wearing, A.H. and Burgess, L.W.** 1977. Distribution of *Fusarium roseum* "Graminearum" Group I and its mode of survival in Eastern Australian wheat belt soils. *Transactions of the British Mycological Society*. **69**, 429-442.
- West, G.** 1975. Comparative pathogenicity of root parasites to wheat seedlings. *Transactions of the British Mycological Society*. **64** (1), 43-53.
- Wollenweber, H.W.** 1931. *Fusarium - Monographie*. Berlin: Julius Springer. pp516.
- Wong, L.S.L., Tekauz, A., Leisle, D., Abramson, D. and McKenzie R.I.H.** 1992. Prevalence, distribution and importance of *Fusarium* head blight in wheat in Manitoba. *Canadian Journal of Plant Pathology*. **14**, 233-238.

Zadoks, J.C., Chang, T.T. and Konzak, C.F. 1974. A decimal code for the growth stages of cereals. *Weed Research*. 14, 415-421.

APPENDICES

Appendix 1 Degrees of freedom, F ratio and probability values for main factors and interactions in the study of the effects of seed contamination and fungicide seed treatment on *Microdochium nivale* seedling blight of wheat.

Factor	D of F	Emergence		Rate of emergence (1/days)		Coleoptile length (mm)		Shoot length (mm)		Root length (mm)		Seedling dry weight (g)	
		F	P	F	P	F	P	F	P	F	P	F	P
<i>M. nivale</i> contamination (Cont.)	4	54.97	<0.0001	4.53	0.0029	27.35	<0.0001	11.09	<0.0001	2.81	0.0331	34.13	<0.0001
Fungicide seed treatment (Fung.)	5	102.3	<0.0001	10.05	<0.0001	101.9	<0.0001	15.45	<0.0001	8.69	<0.0001	46.8	<0.0001
Cont. x Fung.	20	19.42	<0.0001	1.39	0.1626	3.46	<0.0001	2.55	0.0028	1.29	0.2244	9.19	<0.0001
Residual	60												

Appendix 2 Degrees of freedom, F ratio and probability values for main factors and interactions in the study of the effects of seed contamination, fungicide seed treatment and temperature on *Microdochium nivale* seedling blight of wheat.

Factor	D of F	Emergence			Rate of emergence			Coleoptile length			Coleoptile infection			Disease index		
		F	P		F	P		F	P		F	P		F	P	
<i>M. nivale</i> contamination (Cont.)	1	N/A	N/A		0.29	0.596		294.1	<0.0001		N/A	N/A		N/A	N/A	
Fungicide seed treatment (Fung.)	4	17.90	<0.0001		7.35	<0.0001		92.24	<0.0001		15.84	<0.0001		8.65	<0.0001	
Temperature (Temp.)	3	15.69	<0.0001		278.2	<0.0001		124.5	<0.0001		22.14	<0.0001		60.6	<0.0001	
Cont. x Fung.	4	N/A	N/A		3.56	0.0088		14.20	<0.0001		N/A	N/A		N/A	N/A	
Cont. x Temp.	3	N/A	N/A		2.07	0.1076		21.35	<0.0001		N/A	N/A		N/A	N/A	
Fung. x Temp.	12	3.22	0.0013		1.60	0.0995		4.074	<0.0001		3.59	0.0005		1.44	1.176	
Cont. x Fung. x Temp.	12	N/A	N/A		0.74	0.7048		2.680	0.0032		N/A	N/A		N/A	N/A	
Residual		60			120			120			60			60		

Appendix 3 F ratio & P values for stem-base infection data from trial sites No. 1 and 2.

Factor	D of F	Plants infected by <i>M. nivale</i> at GS				Plants infected by <i>F. culmorum</i> at GS			
		10-11	30-31	45	75	10-11	30-31	45	75
Site No. 1									
Fungicide seed treatment	5	F	25.66	N/A	0.685	N/A	-	N/A	0.699
		<i>P</i>	<0.00001	N/A	0.6423	N/A	-	N/A	0.632
Site No. 2									
Fungicide seed treatment	5	F	13.015	1.277	3.837	6.442	-	3.402	2.565
		<i>P</i>	0.0001	0.3243	0.0194	0.0022	-	0.0298	0.7983
Residual	15								